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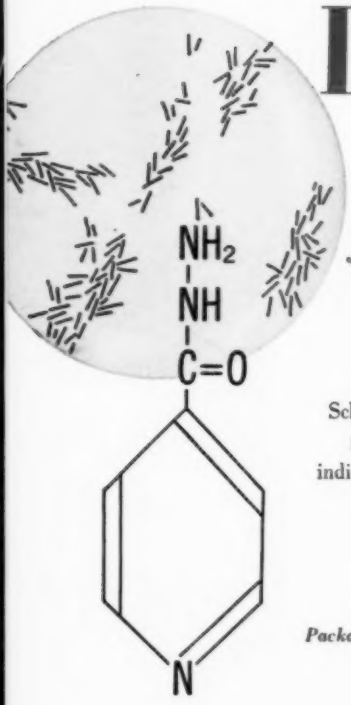

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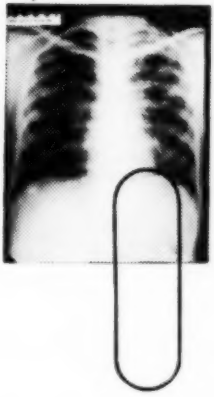

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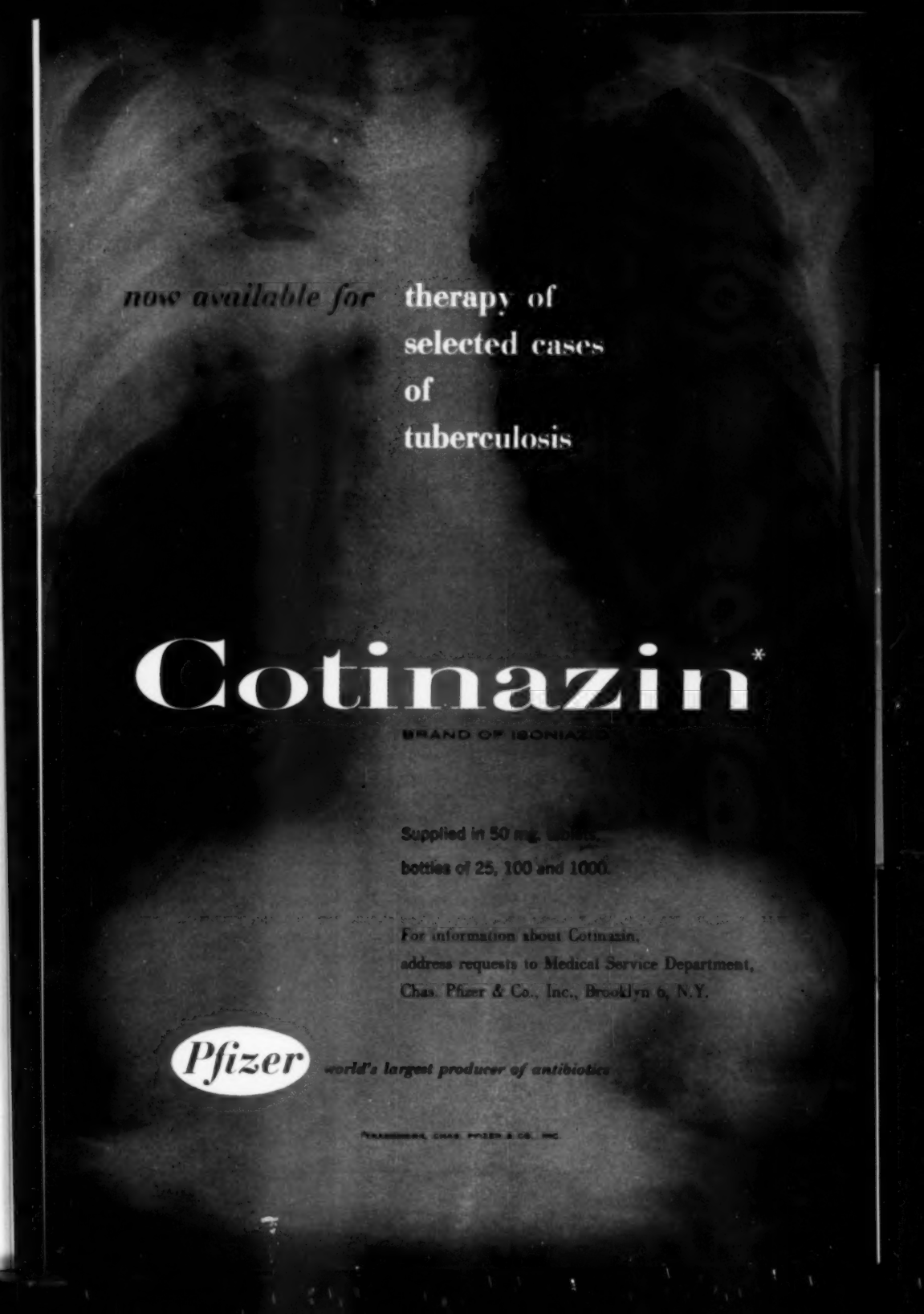
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VOLUME 66 *August, 1952* NUMBER 2

THE ACTION OF ISONIAZID (ISONICOTINIC ACID HYDRAZIDE) ON INTRACELLULAR TUBERCLE BACILLI[1]

G. B. MACKANESS[2] AND N. SMITH

(Received for publication May 20, 1952)

INTRODUCTION

In tuberculosis there exist several obstacles to effective chemotherapy. These recently have been discussed at length by Florey (1). The intracellular location of infecting tubercle bacilli is one of several factors which may play a significant part in determining the response of the disease to chemotherapeutic agents. Living bacilli segregated in macrophages and epithelioid cells, which may not be penetrated by substances in tissue fluids, may persist during therapy and account for the failure of otherwise potent antibacterial agents to cure experimental tuberculosis in animals.

Until recently there were no precise data concerning the activity of antituberculous drugs on intracellular bacilli. A method has been described (2) for determining the sensitivity to drugs of intracellular bacilli *in vitro*. In view of the current interest in isoniazid (isonicotinic acid hydrazide[3]) the findings of some experiments with this drug are presented, together with some comparative data from previous work (3).

Previous Observations

When isolated mammalian macrophages, kept at 37°C., are infected with a virulent strain of *M. tuberculosis*, the intracytoplasmic bacilli commence to multiply after approximately forty-eight hours of incubation. Provided a fully virulent strain is used, the onset of growth is followed almost immediately by death and lysis of the macrophage host cell. Uninfected macrophages will remain alive and in an apparently normal condition for long periods, and can be observed in a healthy motile state for several weeks when kept under suitable conditions.

The proliferation of intracellular tubercle bacilli and the lysis of the host cell which attends it can be wholly reversed by a sufficient concentration of streptomycin. For a virulent bovine strain (*Branch*), the concentration of streptomycin which must be

[1] From the Sir William Dunn School of Pathology, Oxford, England.

[2] Research Fellow of the Australian National University, Canberra.

[3] Supplies of isoniazid (Pycazide) were made available through the courtesy of Herts Pharmaceuticals, Ltd., Welwyn Garden City, Hertfordshire, U. K.

maintained continuously in the ambient culture fluid to achieve this effect was 10.0 γ per ml. The corresponding concentration for intracellular bacilli of the human strain H37Rv was 25 γ per ml. Cultures of both strains in Dubos-Davis medium were effectively inhibited by a concentration of streptomycin of 0.6 γ per ml.

In table 1 the activities of several agents in Dubos-Davis medium are compared with the activities found against intracellular bacilli of the bovine strain *Branch*. In most instances the respective concentrations of each substance necessary to inhibit the intracellular growth of this strain were considerably higher than those required in the orthodox bacterial cultures.

By transferring macrophage cultures after a period of seven days of incubation to an oleic-acid–albumin–agar medium, it was found that a concentration of streptomycin of 25 γ per ml. had not killed all intracellular bacilli of the H37Rv strain and that an occasional viable bacillus still remained in macrophages receiving 50 γ per ml.

TABLE 1

BACTERIOSTATIC ACTIVITY OF DRUGS AGAINST M. TUBERCULOSIS (BRANCH) GROWN IN DUBOS-DAVIS MEDIUM AND IN LIVING MACROPHAGES

DRUG	LOWEST INHIBITORY CONCENTRATION (γ PER ML.)	
	Dubos-Davis* Cultures	Macrophage Cultures
Isoniazid	0.03	0.05
Streptomycin	0.6	10.0
Terramycin	12.5	12.5
Neomycin B	1.56	25.0
Viomycin	6.25	100.0
PAS	1.56	>100.0
Nisin	5–10	No activity
Penicillin	—	No activity

* Dubos-Davis cultures were read at the end of fourteen days.

MATERIALS AND METHOD

The method is described in detail elsewhere (3) and is indicated here only in outline.

Macrophages obtained from the peritoneal cavity of rabbits following the instillation of liquid paraffin (4) were parasitized with the virulent bovine strain *Branch* or with the human strain H37Rv of *M. tuberculosis* grown in liquid Tween[1]-albumin medium. Parasitism was effected by centrifuging a suspension of macrophages in fresh rabbit serum to which had been added a highly dispersed suspension of the tubercle bacilli in Dubos-Davis medium. The macrophage populations obtained in this way contained more than 70 per cent of infected cells.

A solution containing 40 γ of isoniazid per ml. in distilled water was sterilized by passage through a sintered glass filter. From it, serial dilutions were prepared in Tyrode's solution. These were incorporated in the final nutrient medium,

[1] Tween 80 is the proprietary name for the polyoxyethylene derivative of Sorbitan monooleate, obtained from the Atlas Powder Co., Wilmington, Delaware.

which consisted of Tyrode's solution containing 25 per cent of serum from a fasted rabbit. Fresh medium, prepared from fresh solutions, was used at each change of medium. This was made after twenty-four hours of incubation and subsequently at forty-eight-hour intervals. The macrophages were kept under direct observation in special culture chambers.

RESULTS

Activity of Isoniazid in Dubos-Davis Medium

Grown in Dubos-Davis medium, the two strains, H37Rv and *Branch*, were inhibited respectively by concentrations of isoniazid of 0.06 and 0.03 γ per ml. for a period of fourteen days. After this time, the behavior of the two strains

TABLE 2

INHIBITION BY ISONIAZID OF TWO STRAINS OF M. TUBERCULOSIS, BOVINE (BRANCH) AND HUMAN H37Rv, GROWN IN DUBOS-DAVIS MEDIUM, AND READ AT INTERVALS OF FOUR DAYS

DAYS OF INCUBATION	CONCENTRATION OF ISONIAZID (γ PER ML.)								
	1.0	0.5	0.25	0.125	0.06	0.03	0.015	0.007	Cont.
Bovine strain *Branch*									
4	−	−	−	−	−	−	−	+	+
8	−	−	−	−	−	−	−	+	+
12	−	−	−	−	−	−	+	+	+
16	−	−	−	−	−	−	+	+	+
20	−	−	−	−	−	−	+	+	+
24	−	−	−	−	−	−	+	+	+
Human strain H37Rv									
4	−	−	−	−	−	−	−	+	+
8	−	−	−	−	−	−	+	+	+
12	−	−	−	−	−	±	+	+	+
16	±	±	±	±	±	+	+	+	+
20	+	+	+	+	+	+	+	+	+
24	+	+	+	+	+	+	+	+	+

was consistently different. As will be seen in table 2, after prolonged incubation, growth appeared in H37Rv cultures in concentrations of isoniazid ranging at least as high as 1.0 γ per ml. On the other hand, attempts have failed to recover viable organisms from inhibited cultures of the *Branch* strain by centrifuging the original inoculum of bacilli and re-suspending it in fresh Dubos-Davis medium free of the inhibitory agent.

Bovine Strain (Branch) in Macrophage Cultures

In the first titration against intracellular bacilli, macrophage cultures infected with the bovine strain were treated with decimal dilutions of isoniazid ranging in concentration from 1.0 mg. per ml. to 0.01 γ per ml. Control cultures

of macrophages were prepared with and without bacilli. Isoniazid had a relatively low toxicity for macrophages, which survived for two days in the presence of 1.0 mg. per ml. and appeared quite unaffected by lower concentrations. Control-infected cultures disintegrated on the third day of incubation; it was only on the fifth day that signs of degeneration appeared in any of the drug-treated cultures, and these amounted to mild changes in macrophages of cultures containing 0.01 γ per ml. In cultures fixed and stained at this time, growth of the intracellular bacilli had been totally inhibited by a concentration of isoniazid of 0.1 γ per ml.

The results can be seen in figure 1 in which the coccobacillary form of the resting bacterial cell of this strain should be noted. Most of the macrophages treated with 0.01 γ per ml. contained short unaltered bacilli similar to those illustrated in figure 1; but an occasional cell harbored one or several elongated forms, some of which were excessively long (figure 2).

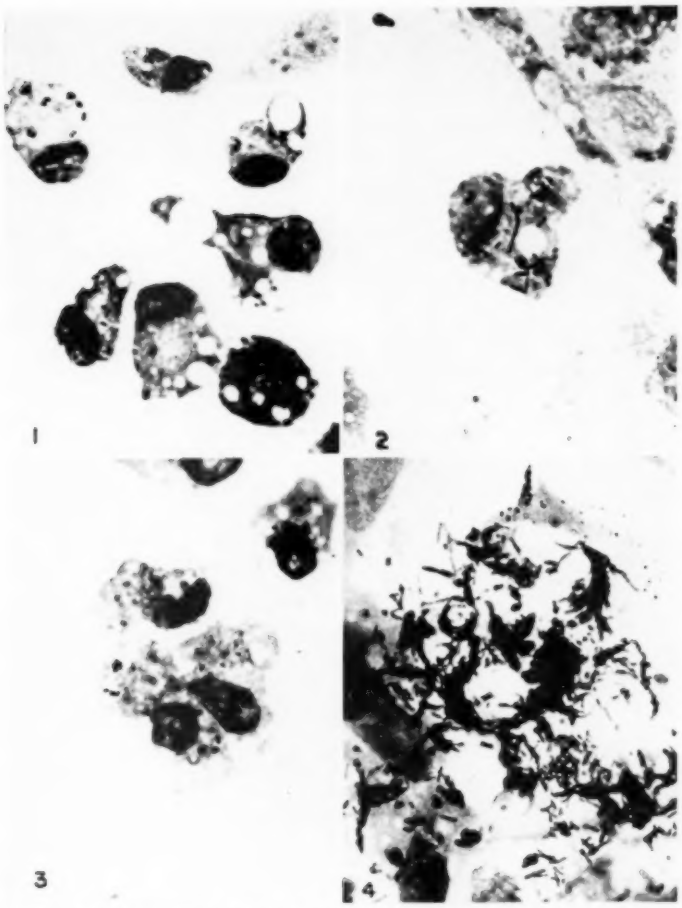
The morphologic change in the bacilli grown in the presence of 0.01 γ of isoniazid per ml. was indication that inhibition had been only partial at this concentration. Three cultures containing 1.0, 0.1, and 0.01 γ of isoniazid per ml. were washed free from the drug and reincubated in medium free from the inhibitory agent. After three days more, the macrophages degenerated in the culture which had previously contained 0.01 γ per ml. Those which had received 0.1 and 1.0 γ per ml. remained normal and healthy in appearance. When fixed and stained, no growth of bacilli could be detected in the latter cultures (figure 3). The death of the macrophages previously treated with 0.01 γ per ml., however, was attributable to the unrestrained growth of the tubercle bacillus (figure 4) which was comparable with that seen in infected control cultures of equivalent age.

In further titrations, intracellular bacilli of the *Branch* strain were completely inhibited for at least eleven days by a concentration in the culture medium of 0.05 γ of isoniazid per ml.

Human Strain H37Rv

The H37Rv strain used in these experiments differed from that of the previous study (3), in which it was shown that fully virulent bacilli of this strain are so highly cytotoxic for isolated macrophages that they are not entirely suitable as a test organism. The present strain had been kept at room temperature on Dorset egg medium. When subcultured, it grew profusely in Dubos-Davis medium, and after two transfers was used as a ten-day-old culture to infect the macrophages. In macrophage cultures it behaved as does the vole bacillus or BCG, multiplying very slowly within the host cells and without evident toxicity for them. Infected macrophages, instead of undergoing lysis after a short period of incubation, continued in a healthy state, many becoming greatly distended by the masses of bacilli which they eventually contained (figure 5). This response suggested that the strain had undergone some attenuation of virulence.

This strain of *M. tuberculosis*, when growing as an intracellular parasite, was



FIGS. 1 and 2. Macrophages infected with the *Branch* strain of *M. tuberculosis* from a culture series treated with decimal dilutions of isoniazid for five days. Ziehl-Neelsen-methylene blue (× 1500).

1. Absence of bacillary growth in the presence of 0.1 γ of isoniazid per ml.

2. Macrophages containing elongated bacillary forms in the presence of 0.01 γ of isoniazid per ml.

FIGS. 3 and 4. Macrophages from parallel cultures to those in figures 1 and 2, washed free from isoniazid and reincubated in medium free from this agent for three days.

3. Absence of bacillary growth following withdrawal of the drug at a concentration of 0.1 γ of isoniazid per ml.

4. Destruction of macrophages and overgrowth by bacilli following withdrawal of the drug at a concentration of 0.01 γ of isoniazid per ml.

also inhibited by isoniazid at a concentration of 0.05 γ per ml. Unproliferated bacilli which had been inhibited for five days by this concentration can be seen within the macrophages of figure 6. It must be pointed out that bacilli of this strain do not assume a coccobacillary form in the resting state. The marked morphologic change which accompanies growth of the *Branch* strain is therefore not seen. In the presence of 0.025 γ of isoniazid per ml., however, multiplication of bacilli was detected in macrophages after the same period because of the pronounced "cord" formation which had taken place in them (figure 7).

On the seventh day, when most cells of the infected controls were crammed with proliferated bacilli (figures 5 and 8), there was still no evidence of growth of bacilli in the presence of 0.05 γ of isoniazid per ml. (figure 9). At this time two

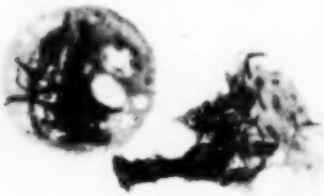
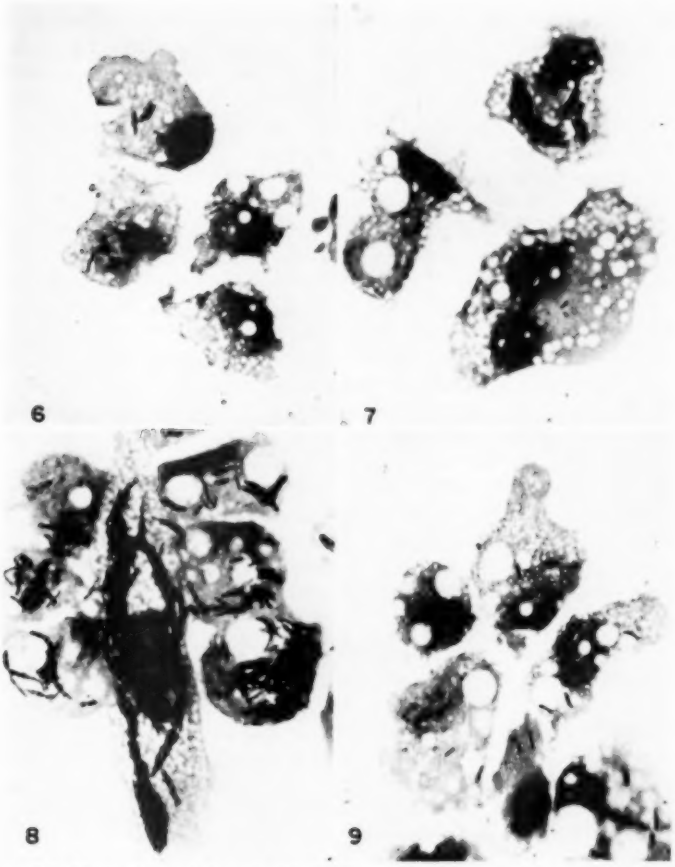


FIG. 5. Macrophages infected with H37Rv strain from a series of cultures treated with isoniazid. Ziehl-Neelsen–methylene blue (× 1500). Macrophages crammed with bacilli after seven days' incubation, showing the extent of bacillary proliferation without damage to the host cell (*see* text). The macrophages of figure 6 give an indication of the initial level of parasitism.

cultures containing, respectively, 0.1 and 0.05 γ of isoniazid per ml. of culture medium were washed free of the inhibitory agent, and the macrophages were allowed to dry in contact with the coverslip. They were then covered with a thin layer of oleic-acid–albumin–agar medium, sealed, and reincubated. Microcolonies of bacilli appeared among the remnants of almost every macrophage of both cultures after four to six days of incubation. It is apparent that intracellular bacilli had survived for a period of seven days in the presence of fully bacteriostatic concentrations of isoniazid.

DISCUSSION

Isoniazid differs from most of the antituberculous drugs which have been tested by this method in that its activity is not significantly lowered by an



FIGS. 6-9. Macrophages infected with H37Rv strain from a series of cultures treated with isoniazid. Ziehl-Neelsen-methylene blue (× 1500).

6. Absence of bacillary growth in five days in the presence of 0.05 γ of isoniazid per ml.

7. Early but definite "cord" formation in one macrophage after five days in the presence of 0.025 γ of isoniazid per ml.

8. Control-infected culture after seven days to show the extent of bacillary proliferation. The field contains a fibroblast filled with long cords of bacilli. From the same preparation as that of figure 5.

9. Absence of bacillary growth in the presence of 0.05 γ of isoniazid per ml. after seven days (compare figures 5 and 8).

intracellular location of the tubercle bacillus. It is possible to conclude, therefore, that the drug enters freely into cells and exerts its full bacteriostatic ac-

tion in this environment. In this it has an advantage over streptomycin which suffers a considerable reduction in activity against intracellular bacilli, so that it probably does not prevent their multiplication *in vivo* at the concentrations which can be sustained therapeutically. A similar view has been expressed by Dennis, Goble, Berberian, and Frelih (5) from their experience with infection in the Syrian hamster.

The present study has revealed a difference in response of the two strains of *M. tuberculosis* used. In Dubos-Davis medium, the bovine strain gave a stable endpoint and could not be recovered in a viable form after fourteen days' exposure to minimal or nearly minimal inhibitory concentrations of the drug. The H37Rv strain, however, did not give a stable endpoint and, when incubation was continued, growth appeared in concentrations at least as high as 1.0 γ of the drug per ml. Steenken and Wolinsky (6) record the same finding and report that the growth which appears in concentrations as high as 25 γ per ml. is due to bacilli which have an increased resistance to the drug.

The above difference of the two strains is to some extent paralleled by their behavior in macrophage cultures. Bacilli of the *Branch* strain appear to have been killed within macrophages by seven days' exposure to not more than twice the minimal inhibitory concentration of isoniazid. No such bactericidal action was found for the H37Rv strain under the same conditions. Higher concentrations of the drug have not, however, been tested.

It seems possible to conclude that the behavior of intracellular bacilli towards isoniazid is not different from their behavior in ordinary bacterial cultures.

SUMMARY

Living isolated rabbit macrophages have been used as a medium for the propagation of mammalian tubercle bacilli growing as intracellular parasites. Of those tested, most antituberculous drugs showed a marked reduction in activity against intracellular bacilli. Thus, 25 γ of streptomycin per ml. are required to prevent growth of the H37Rv strain in this location. Isoniazid, however, is equally active against intracellular bacilli and those grown in Dubos-Davis medium. It produced inhibition of the same strain at a concentration of 0.05 γ of drug per ml.

SUMARIO

La Acción de la Isoniacida (Hidracida del Acido Isonicotínico) sobre los Bacilos Tuberculosos Intracelulares

Como medio para la propagación de bacilos tuberculosos mamíferos que crecían como parásitos intracelulares usáronse macrófagos vivos aislados de conejos. La mayor parte de las drogas antituberculosas ensayadas revelan decidida reducción de su actividad contra los bacilos intracelulares. Por ejemplo, se necesitan 25 γ de estreptomicina por ml. para impedir el desarrollo de la cepa H37Rv en dicha localización. Sin embargo, la isoniacida es igualmente activa contra los bacilos intracelulares y contra los cultivados en el medio de Dubos-Davis, y produjo inhibición de la cepa mencionada a una concentración de 0.05 γ de droga por ml.

RESUMÉ

L'action de l'isoniazid (hydrazide de l'acide isonicotinique) sur les bacilles tuberculeux intracellulaires

Des macrophages vivants isolés, provenant de lapins, ont été utilisés comme milieu de reproduction de bacilles tuberculeux de mammifères se développant comme parasites intracellulaires. La plupart des médicaments antituberculeux, parmi ceux soumis aux tests permettent d'observer une réduction marquée d'activité vis à vis des bacilles intracellulaires. C'est ainsi qu'il faut 25 γ de streptomycine par ml. pour prévenir de développement de la souche H37Rv située dans la cellule. Toutefois, l'isoniazid se montre également actif contre les bacilles intracellulaires et contre ceux cultivés dans le milieu Dubos-Davis. Ce produit, à la concentration de 0,05 γ par ml., détermine l'inhibition de cette même souche.

Acknowledgments

The authors wish to thank Professor H. W. Florey for his interest and criticism during the execution of this work. The careful technical assistance of Messrs. A. A. Argyle and D. Etheridge is gratefully recognized.

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GUIDED SYMPHYSIS[1,2]

Functional Prophylaxis in Exudative Pleurisy and Pneumothorax Fluid

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INTRODUCTION

Because of the recent advances in thoracic surgery and the development of specific antimicrobial agents, the possibilities for active therapy in pulmonary tuberculosis are now governed less by the toxicity and localization of the disease than by the functional condition of the heart and lungs. In the great majority of patients requiring surgical therapy, the disease can be rendered relatively stable with preoperative treatment, and postoperative spread of disease represents a relative rarity (1). Nevertheless, the presence of such conditions as extensive emphysema or grave impairment of ventilatory function as a result of pleurisy imposes strict limitations on surgical therapy. Even if the tuberculous process is controlled by pulmonary resection, transthoracic cavity drainage, and similar procedures, these patients remain respiratory and circulatory "cripples" with a greatly shortened expectation of life.

Emphysema, the commonest type of *parenchymal insufficiency* (2), and impaired ventilation, *ventilation insufficiency*, together with their secondary cardiac effects are, in fact, the commonest causes of functional insufficiency in tuberculous subjects.

Although the prevention of emphysema is not yet possible, ventilatory insufficiency is, to a certain extent, preventable. This applies particularly to that form resulting from pleurisy, in which the chief impairment of function is reduced diaphragmatic mobility, i.e., *diaphragmatic insufficiency*.

In mild cases of this form of insufficiency, there is insignificant impairment of diaphragmatic mobility, and the lower part of the costophrenic sulcus is usually obliterated. In more severe cases, the sulcus is totally obliterated and the diaphragmatic plane is roughly horizontal on inspiration in the erect posture. Mobility is relatively good. In the gravest cases, the diaphragm is firmly adherent to the chest wall and mobility is very slight.

Adhesion of the pleural membranes, with consequent disturbance of the gliding movement that is essential for uniform pulmonary expansion, must perforce be detrimental to ventilation. Some parts of the lung are then better ventilated than others. If the adhesions are grossly fibrous, the movements of the ribs will be reduced and ventilation still further impaired. The damage to diaphragmatic mobility, however, would seem to be the dominant factor in this condition.

[1] From the Medical Tuberculosis Division II, Söderby Hospital, Uttran; and Karolinska Institute, Stockholm, Sweden.

[2] This study has been carried out with the help of a grant from the Swedish National Association against Tuberculosis.

When assessing diaphragmatic function, it is most important to study the movements of the posterior part. Not infrequently one sees extensive adhesion and poor mobility frontally and laterally while the posterior part of the diaphragm shows good mobility.

Causes of Pleural Symphysis

Pleural obliteration occurring in pulmonary tuberculosis is most commonly caused by:

(*a*) pleurisy, usually with effusion
(*b*) pneumothorax fluid, or
(*c*) postoperative pleurisy and pleural effusion

Exudative pleurisy is by far the most common cause of pleural symphysis, and attempts to prevent adhesion by replacement of fluid with air or by intrapleural heparin therapy have not been successful.

In regard to preventive measures against pleurisy complicating pneumothorax therapy, much headway has already been made by improved case selection. It is now recognized that the risk of fluid formation is greatest in cases with acute exudative pulmonary lesions or incomplete pneumothorax. Postponement of induction until chemotherapy has decreased the activity of the disease and abandonment of unsatisfactory pneumothoraces have brought about a decline in the frequency of pneumothorax fluid. With the progress of thoracic surgery, many of the cases formerly submitted to extensive adhesion section, with high effusion risk, are now treated instead by measures such as extrapleural pneumothorax or thoracoplasty.

The last-named procedures, however, are also not infrequently attended by pleural complications, which generally arise from perforation into a free pleural cavity during the operation, resulting in an undesired "pneumothorax mixte." Postoperative pleural effusion is highly unfavorable in such cases because respiration is fairly shallow in character, due to pain, and the risk of high diaphragmatic adhesion is consequently increased. In order to avoid this complication, attempts are often made preoperatively to bring about pleural adhesion by means of artificially produced pleurisy. The eventual site of such adhesions cannot be foretold with accuracy; and if diaphragmatic mobility is considerably impaired in the process, the price paid for symphysis will have been too high.

The great practical importance of the problem of postpleuritic ventilation insufficiency has been clearly demonstrated by the bronchospirometric investigations of Pinner, Leiner, and Zavod (3) and Bruce (4). Postpleuritic pleural fibrosis may impair ventilation to such a degree that oxygen uptake in the lung becomes practically impossible.

PLAN OF INVESTIGATION

The present study covers the period from the autumn of 1949 to the spring of 1951 and includes all but the mildest cases of exudative pleurisy and pneumothorax fluid encountered during that period.

Nineteen cases of exudative pleurisy with no roentgenographically demonstrable parenchymatous lesions (in this group, 4 patients with artificially produced pleurisy are included), 23 cases of pleurisy complicating pneumothorax therapy, and 6 cases of postoperative serosanguineous effusions are classed as "recent" pleurisies. This means that therapy was instituted less than three months after the appearance of the effusion. Therapy was also attempted in 17

patients with effusions which had been present for more than three months when treatment was begun. These are designated as "older" pleurisies. The average period of therapy was sixty-four days in the recent cases and eighty-seven days in the older cases (table 1).

Outline of Therapy

Adhesion of the diaphragm to the chest wall postulates that the caudal part of the lower lobe of the lung leaves its original position in the sulcus, usually forced out by pleural fluid. If the lung is assisted to remain in its proper position while the fluid is organized and absorbed, high adhesion of the diaphragm to the chest wall, with its ill effects on function, will be prevented. Frequent aspiration of the fluid, without entry of air into the pleural cavity, will help toward this end. Weekly or biweekly aspiration is generally adequate. Intrapleural injection of streptomycin (0.5 to 1 gm.) daily or on alternate days, combined with oral administration of para-aminosalicylic acid and parenteral streptomycin, hastens the healing process.

Posture therapy: The level of the diaphragm is altered when the lateral posture is adopted (5, 6). The lower leaf rises into the thorax pushing the lung before it (figure 1). If a patient

TABLE 1

THE DURATION OF THERAPY FOR "GUIDED SYMPHYSIS"

TYPE OF EFFUSION	RECENT EFFUSIONS (PRESENT FOR LESS THAN THREE MONTHS WHEN THERAPY WAS BEGUN)		OLDER EFFUSIONS (PRESENT FOR MORE THAN THREE MONTHS WHEN THERAPY WAS BEGUN)	
	Number of patients	Mean period of therapy	Number of patients	Mean period of therapy
		Days		*Days*
Exudative pleurisy	19	67	2	101
Hydropneumothorax	23	64	15	85
Postoperative pleural effusion	6	59	—	—
Total	48	64	17	87

with pleurisy lies on the affected side for any length of time, the tendency to obliteration may cause the diaphragm to adhere high on the chest wall, resulting in considerable impairment of its mobility. This also applies in some degree to the supine posture, in which the posterior sulcus may become partially obliterated. The patient with pleurisy, therefore, should avoid lying on the affected side and on the back.

While the lower leaf of the diaphragm moves up into the thorax when the subject adopts the lateral posture, the upper leaf is drawn down towards the abdomen, and the costophrenic sulcus on this side gapes open. If the affected side lies uppermost, therefore, the sulcus will receive the lung when the fluid is aspirated, and further intimate contact between the diaphragm and the chest wall will be avoided during the critical phase of obliteration. The detrimental effects on the posterior part of the sulcus may also be avoided, particularly if the patient lies turned somewhat forward toward the prone position.

Breathing exercises: The effect of posture therapy may be enhanced by breathing exercises. Repeated, deep respirations prevent undesirable adhesion of the diaphragm to the chest wall and the formation of pulmonary adhesions on sites unfavorable for function. The patient is therefore instructed to take about fifteen deep breaths each hour.

Physiotherapy also includes abdominal breathing, so that the greater part of the ventilatory movements is performed by the diaphragm and not by the thoracic cage.

When performing breathing exercises the patient should, of course, adopt those postures in which the diaphragmatic movements bring about maximum opening of the costophrenic sulcus, viz., the lateral posture with the affected side uppermost, and forward and backward rotation from this posture.

At a later stage in therapy the patient is taught to breathe selectively in order to increase the mobility of the ribs on the affected side. This is most easily achieved by breathing against resistance, e.g., a band held round the thorax.

Should breathing exercises be begun in the initial stage of pleurisy, when the patient is still febrile, or should one wait until the temperature has become normal? In the hundred or more patients treated up to the present, therapy was begun as soon as the patient entered the hospital, regardless of the stage of the disease, and no untoward effects have been observed. Naturally, some caution in the performance of breathing exercises is advisable in the most acute phase of the illness, but posture therapy need not be modified and may be instituted without delay.

FIG. 1. The diaphragm of a healthy subject in the left lateral posture. The lower leaf has risen into the thorax and the upper has moved away from the lateral wall of the chest.

Electrophrenic respiration: It sometimes happens that a patient cannot understand fully the instructions for breathing exercises or is physically unable to cooperate. For instance, there may be reflex immobility of the diaphragm in the initial phase of pleurisy. In such cases diaphragmatic movements can be artificially produced by stimulation of the phrenic nerve with the electrophrenic respirator devised by Sarnoff and associates (7). An intermittent, rhythmically rising and falling faradic current is applied to the site of the phrenic nerve in the neck. The current can be regulated to produce contractions of a desired

strength. The same strength of current may cause contractions varying greatly in intensity in different patients. Assessment of the effect of the stimulation is made by fluoroscopic observation or by placing the hand on the patient's upper abdomen (figure 2). The only discomfort felt by the patient during this procedure is slight irritation at the site of electrode application.

Up to the present, each treatment has consisted of no more than fifteen contractions. But treatment has been repeated daily. As experience of the method increases, it is believed that it will be possible to exceed this number of contractions. This should be particularly

FIG. 2. The diaphragmatic excursion produced by the electrophrenic respirator (electrode applied to the right phrenic nerve). The subject is a healthy person in the supine posture. (Taken with the double-exposure grid according to Birath and Källqvist).

valuable in patients who have passed the acute stage of pleurisy, but who cannot perform effective breathing exercises unaided, and in the treatment of postoperative pleural complications.

In the writer's opinion, electrophrenic respiration is a very valuable adjunct to the other methods for preventing impairment of function after pleurisy, but it is too early as yet to formulate detailed indications and instructions for its use.

Treatment of pneumothorax fluid: The program of therapy in pleurisy complicating artificial pneumothorax is essentially the same as in simple pleurisy with effusion.

A large effusion occurring in the course of ambulant pneumothorax therapy, such as "primary effusion," is considered at Söderby a clear indication for hospitalization. The functional prognosis is greatly improved if the patient is admitted to the hospital where the effusion can be aspirated regularly and intrapleural antibiotics, physiotherapy, et cetera given.

In the majority of patients treated with artificial pneumothorax, the tuberculous lesion is situated in the apical and posterior segments of the upper lobe, and selective apical collapse is generally obtainable by adhesion section. The lower lobe may thus be kept expanded. If pleural effusion occurs, however, the lower lobe is compressed and in time becomes covered with a fibrous mantle that hinders re-expansion. The object of aspiration is to counteract this compression. Refills of air at suitable intervals will maintain the apical collapse while the lower lobe is allowed to adhere to the diaphragm and chest wall. These refills should be small and frequent, usually once weekly, to prevent tearing of the loose fibrinous adhesions on the chest wall that will gradually fix the lower lobe in the desired position.

This technique may sometimes be associated with the risk that the lung will adhere more extensively than is desired and obliteration will continue, even when the amount of air given at refills is increased. Although the risk is small, it must be stressed that careful observation and timing are very important for its avoidance.

Contraindications: One possible contraindication to the treatment is very acute pleurisy, in which the increased thoracic movements might aggravate the condition. It is not an absolute contraindication, however; as stated in the foregoing, treatment may be given provided caution is exercised.

Tension cavities may constitute a relative contraindication, as they may be inflated by the increased movement. Such cavities are now relatively rare, however, thanks to specific antimicrobial agents, and were present in none of the patients in this study.

RESULTS

The results of therapy were assessed in regard to the respiratory movements of the diaphragm and lungs.

The respiratory movements can be registered on double-exposure roentgenograms. With a simple apparatus, the double-exposure grid according to Birath and Källqvist (8), films can be taken that clearly show the respiratory movements of the ribs and diaphragm.[3] This grid has vertical strips of lead 0.5 cm. in breadth and 0.5 cm. apart. Half of the surface of the film is thus covered by the lead strips at each exposure. After the first exposure, the grid is moved sideways the breadth of one lead strip so that the already exposed bands of film are covered and the others left free for the second exposure. On the completed roentgenogram, alternate film strips show the inspiratory picture, and alternate strips show the expiratory. The errors arising from the changed position of the body in relation to the roentgenogram in the movements of respiration are considered to be negligible, provided that the position of the vertebrae is the same on both exposures. To ensure that the patient has made a maximum respiratory effort, fluoroscopic control is necessary. In most cases, however, the absolute degree of respiratory movement is less important than registration of the diaphragmatic contours in both respiratory phases and the assessment of any difference between the two leaves.

[3] After the first publication concerning this grid (8), the present writer discovered that Pollitzer (9) had previously described a similar method.

On posterior-anterior roentgenograms or fluoroscopy, the diaphragm is seen to have a piston-like movement, almost parallel to its own contour. The lateral movements and those of the medial parts show very little difference even in the extremes of respiration (figure 3). Seen laterally, however, the posterior part of the diaphragm has a greater range of movement and is consequently more important for ventilation than the anterior part, where mobility is considerably less (figure 4).

In most cases in the present study, posterior-anterior and lateral double-exposure roentgenograms were taken in maximum inspiration and expiration. In the other cases, fluoroscopic estimation was made from all aspects during deep respiration.

Fifty-four of the 65 patients have been followed for six months and the remainder for three months.

The results have been graded as follows:

0 = No improvement. Diaphragmatic mobility was greatly reduced, and in many cases there was incomplete re-expansion of the lung.
+ = Some improvement of diaphragmatic mobility, but considerable remaining damage. In some cases there was pleural thickening.
++ = Good result, but moderate impairment of diaphragmatic mobility due to adhesion to the chest wall or persistent fluid in the sulcus.
+++ = Very good result, with normal or almost normal contours and mobility of the diaphragm.

In those cases in which therapy was instituted early, the results were generally good. Of the 48 recent cases, good (++) or very good (+++) results were recorded after three months in 40 patients (table 2). The most successful cases were those with pleural effusion resulting from primary tuberculous infection and postoperative effusions. Of 25 such patients, good or very good results were shown in all but one. In this patient, artificial pleurisy had been evoked as treatment for a spontaneous pneumothorax of three years' duration. No improvement was noted for the first six months, but after one year's observation the diaphragmatic movement could be classed as "good" (++).

Poorer results were shown by patients treated for effusion complicating artificial pneumothorax, even when therapy was begun soon after the appearance of the effusion. Of 23 such patients, 7 showed no or only slight improvement after three months. Not infrequently, however, improvement takes place slowly, so that a result classed as "good" after three months can be "very good" after six months. Table 3 shows that 4 of the 7 patients mentioned above improved and 2 of them showed "very good" respiratory mobility without further treatment or with exercises carried out on their own initiative at home. Of the 3 patients who failed to show any improvement, one had an effusion complicating re-expansion of the lung after a long-standing pneumothorax.

It is obvious that, if therapy is not begun until more than three months after the onset of the effusion, the results cannot be so satisfactory. Most of the "old" effusions were present as complications of artificial pneumothorax (table 2). Of 15 such patients, 9 showed no or insignificant improvement after three

FIG. 3. (Left) The normal excursion of the diaphragm and thoracic cage at maximum inspiration and expiration as seen on the posterior anterior roentgenogram. (Taken with the double-exposure grid according to Birath and Kallqvist.)

FIG. 4. (Right) Lateral view of the subject in figure 3

TABLE 2

RESULTS OF "GUIDED SYMPHYSIS"*

(After Three Months' Observation)

RECENT EFFUSIONS (PRESENT FOR LESS THAN THREE MONTHS WHEN THERAPY WAS BEGUN)	0	+	++	+++	TOTAL
Exudative pleurisy	1	—	4	14	19
Hydropneumothorax	3	4	7	9	23
Postoperative pleural effusion	—	—	3	3	6
Total	4	4	14	26	48
OLDER EFFUSIONS (PRESENT FOR MORE THAN THREE MONTHS WHEN THERAPY WAS BEGUN					
Exudative pleurisy	1	—	—	1	2
Hydropneumothorax	6	3	3	3	15
Postoperative pleural effusion	—	—	—	—	—
Total	7	3	3	4	17
Grand total	11	7	17	30	65

* For explanation of classification 0, +, et cetera, see text.

TABLE 3

RESULTS OF "GUIDED SYMPHYSIS"*

(After Six Months' Observation)

RECENT EFFUSIONS (PRESENT FOR LESS THAN THREE MONTHS WHEN THERAPY WAS BEGUN)	0	+	++	+++	TOTAL
Exudative pleurisy	1	—	2	12	15
Hydropneumothorax	3	1	5	11	20
Postoperative pleural effusion	—	—	1	2	3
Total	4	1	8	25	38
OLDER EFFUSIONS (PRESENT FOR MORE THAN THREE MONTHS WHEN THERAPY WAS BEGUN)					
Exudative pleurisy	—	1	—	1	2
Hydropneumothorax	5	—	4	5	14
Postoperative pleural effusion	—	—	—	—	—
Total	5	1	4	6	16
Grand total	9	2	12	31	54

* For explanation of classification 0, +, et cetera, see text.

months (in 2 cases the effusion had developed at pulmonary re-expansion after artificial pneumothorax). After six months, however, 4 of these patients were improved and the remaining 5 were unchanged.

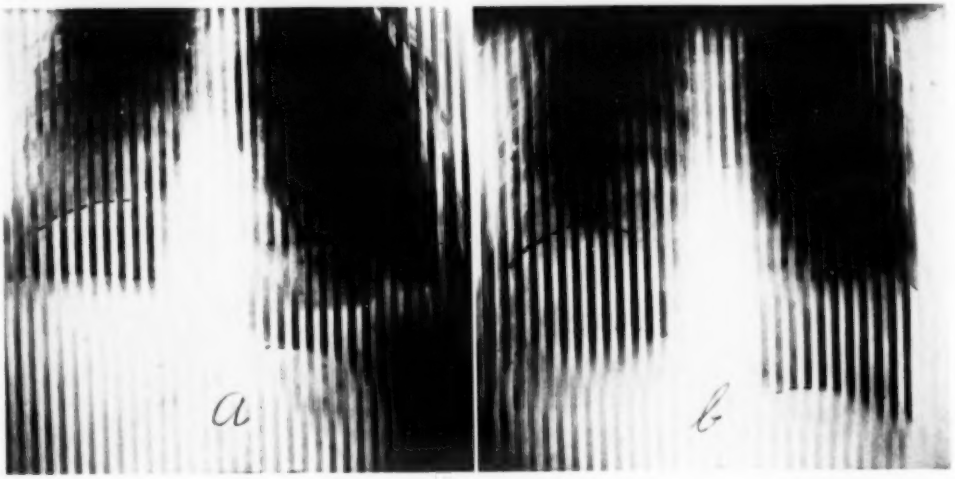

FIG. 5. S-A. D., a 20-year-old male medical student, developed right pleurisy six months prior to admission to the hospital, and for this reason had been given pneumothorax therapy at another institution. (This prophylactic pneumothorax has been practiced chiefly in Sweden and France but now appears to be losing favor.) Ventilation movements were poor on admission to Söderby (film *a*) and the posterior sulcus was completely obliterated. A short time previously, infiltration and cavity had appeared in the left lung, necessitating pneumothorax therapy. The first pneumothorax was promptly abandoned and breathing exercises were commenced. Pleural thickening made pulmonary expansion difficult, but after several months of intensive effort ventilation was so far improved that very little difference could be seen between the two sides (film *b*). The cavity in the left lung closed rapidly under pneumothorax therapy.

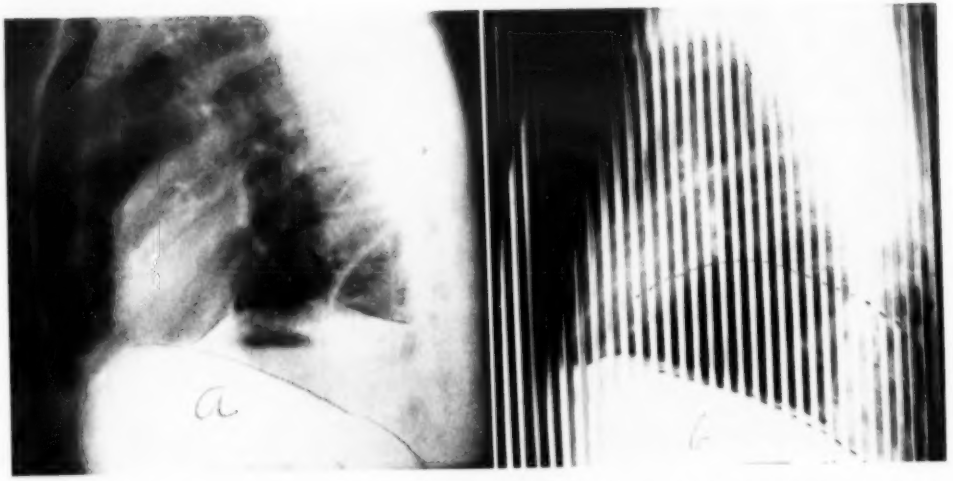

FIG. 6. E. N., a 20-year-old student, developed hilar adenitis in the autumn of 1950 and, shortly thereafter, exudative pleurisy with persistent high fever. He was given para-aminosalicylic acid (PAS) and streptomycin, but no intrapleural therapy. Aspiration and posture therapy were begun immediately and breathing exercises were commenced when the temperature began to fall. The first roentgenograms showed poor peripheral diaphragmatic movement and an obliterated posterior sulcus (film *a*). After treatment, which included electrophrenic respiration, the diaphragmatic mobility was good and the sinus had almost completely reopened (film *b*). The contours of both hemidiaphragms in inspiration position almost cover each other and have to be compared with film *a* where they greatly differ.

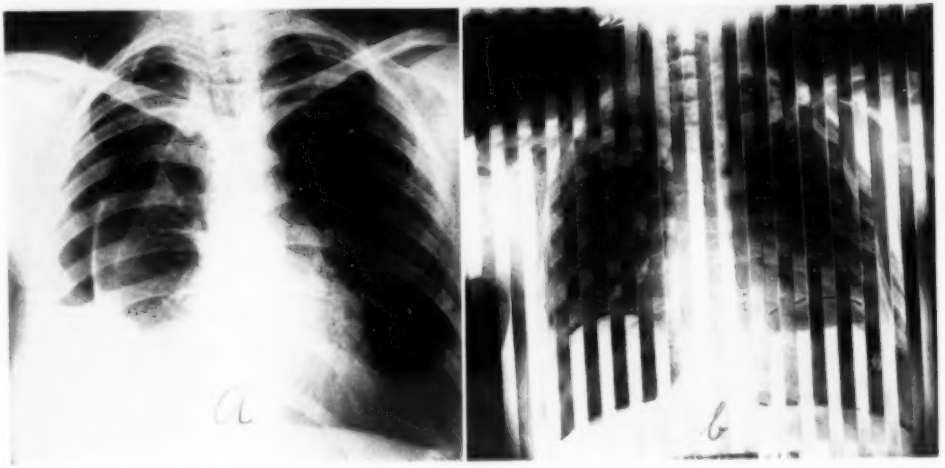

FIG. 7. A. S. was a 27-year-old housewife in whom pleural effusion formed after approximately one year's pneumothorax therapy. When she arrived at the hospital, the base of the lung was compressed (film *a*). There was no choice but to abandon the pneumothorax and give posture therapy and breathing exercises. In spite of the very unfavorable initial conditions, an excellent result was obtained, with completely normal diaphragmatic movements (film *b*). There has been no progression of the lung lesions but, should progression occur, it could be controlled by a small apical thoracoplasty.

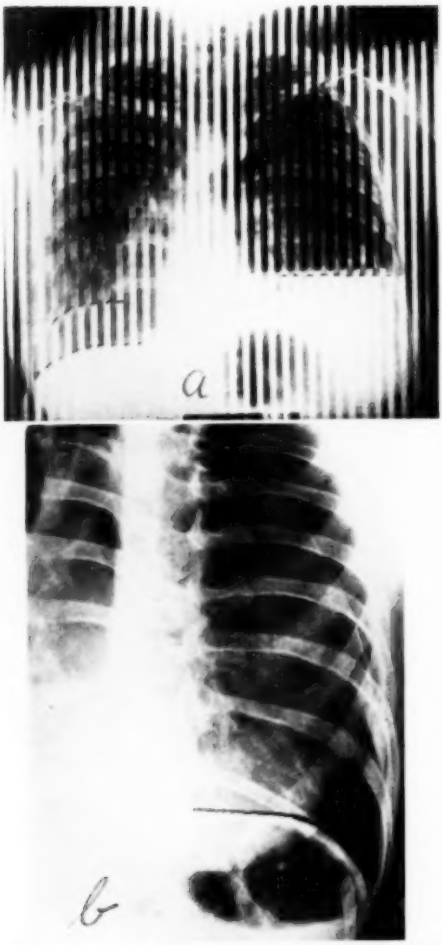

FIG. 8. M. T. was a 30-year-old housewife who developed pleural effusion with pyrexia six months after pneumothorax induction. She was immediately admitted to the hospital, given combined medication with intrapleural PAS, posture therapy, breathing exercises, and aspiration. To begin with, there was complete reflex immobility of the diaphragm (film *a*.) In spite of this and an unusually persistent effusion, the sulcus was kept open and the lung guided out toward the chest wall. The final result was an ideally selective pneumothorax over the upper lobe with no limitation of diaphragmatic movement (film *b*).

DISCUSSION

The advisability of the above-described treatment in an inflammatory disease such as pleurisy is open to the criticism that active movements generally are

FIG. 9. B. M., a 25-year-old housewife, developed effusion following adhesion section approximately one month after pneumothorax induction (film *a*). While the fluid was never very abundant, it was very persistent and displayed a tendency to compress the base of the lung. Routine treatment resulted in re-expansion of the lower lobe and satisfactory return of function (film *b*).

contraindicated in all inflammatory conditions. The treatment, however, can hardly be compared with active-movement therapy, and it is scarcely possible that the few deep respirations performed each hour are more irritating than the approximately 900 hourly respirations of ordinary depth. At any rate, the writer has never observed a relapse of the disease that could be attributed to the treat-

ment. A patient may complain of slight pleural pain after excessive cooperation in his treatment, but the pain quickly disappears when his zeal is suitably dampened.

In the present series, the best results were obtained in patients who showed a constant, cooperative interest in the treatment.

Some obliteration of the posterior sulcus is not infrequently present when treatment is begun or during its initial period after the greater part of the effusion has been reabsorbed (figure 6). Mobility is always considerably reduced at this stage. During the course of therapy, and often even in the ambulant stage after its conclusion (free movement is undoubtedly beneficial), one sees how the sulcus is re-formed and the diaphragmatic contours return more or less to normal (figures 5, 6, 7, 8, and 9). This obliteration is probably caused by a small amount of fluid which persists in the sulcus or loose fibrin adhesions that respond to therapy. The poorest results are shown by patients who develop effusion at re-expansion of a long-standing artificial pneumothorax. In these cases the visceral pleura probably is already thickened and adhesions form between the diaphragm and the chest wall without the occurrence of true pleurisy.

The results obtained have greatly decreased fear of the untoward sequelae of pleural effusion during the course of pneumothorax therapy (figure 8). The occurrence of a mild pleurisy, e.g., after adhesion section, is thus even regarded as an opportunity to achieve adhesion of the lower lobe to the chest wall and diaphragm in a position that will protect pulmonary function from the deleterious effects of any future effusion (figure 8). It is of the utmost importance, however, that treatment should be begun early, as soon as the effusion has appeared. Otherwise, the edges of the lower lobe may "fold in" and it will then be impossible to guide them down to fill the sulcus.

Pre- and post-induction medication with specific antimicrobial agents has eliminated the occurrence of empyema and, if the danger of impaired function can largely be avoided by the measures outlined here, the fear of pneumothorax therapy will have lost its justification.

The treatment has also been used with great success to guide adhesion formation in a few cases of hemopneumothorax. Moreover, it has been used after pulmonary decortication, when it is essential that the re-established ventilation should have the greatest possible efficacy.

SUMMARY

Ventilation insufficiency is one of the commonest causes of pulmonary insufficiency; preventive measures should be directed against the conditions generally responsible for ventilatory impairment, viz., pleurisy with effusion and pneumothorax fluid. The role of the diaphragm in this connection is particularly stressed.

A program of prophylaxis is described and consists of:

1. Adoption of the lateral posture with the affected side uppermost
2. Breathing exercises (abdominal breathing), in certain cases including electrophrenic respiration

3. Frequent pleural aspiration and medication with specific antimicrobial agents

Sixty-five patients were treated with these measures. In 48 cases the effusion was of recent date; of these, 40 showed good or very good ventilatory mobility after an observation period of three months. In 17 patients with effusions of more than three months' duration at the commencement of treatment, the results were less satisfactory. Seven patients showed satisfactory diaphragmatic movements after the same observation period, but function was considerably impaired in 10 cases. In some of these patients, however, improvement was noted after a further three months of observation.

SUMARIO

La ventilación insuficiente es una de las causas más comunes de la insuficiencia pulmonar, de modo que las medidas preventivas deben encaminarse a los estados que en general son causantes de la deficiencia ventilatoria, a saber, la pleuresía con derrame y el hidroneumotórax. Recálcase en particular el papel del diafragma en ese sentido.

El plan profiláctico descrito comprende:

1. Adopción de la postura lateral con el lado afectado más arriba;
2. Ejercicios respiratorios (respiración abdominal) en ciertos casos, comprendiendo la respiración electrofrénica, y
3. Frecuente aspiración y medicación pleural con agentes antimicrobianos específicos.

Sesenta y cinco enfermos fueron tratados con esas providencias. En 48, el derrame era reciente; de ellos, 40 revelaron buena o buenísima movilidad ventilatoria al cabo de un período de tres meses de observación. En 17 enfermos con derrames de más de tres meses de duración al comenzar el tratamiento, el resultado fué menos satisfactorio. Siete enfermos revelaron movimientos satisfactorios del diafragma al cabo del mismo período de observación, pero en 10 la función estaba considerablemente afectada. Sin embargo, en algunos de los últimos notóse mejoría después de otros tres meses de observación.

RESUMÉ

L'insuffisance de la ventilation est une des causes les plus communes de l'insuffisance pulmonaire. Des mesures préventives doivent être dirigées contre les conditions généralement responsables de l'insuffisance de la ventilation, en particulier dans la pleurésie avec épanchement et dans l'exudat du pneumothorax.

A cet égard le rôle du diaphragme est particulièrement mis en évidence.

Un programme de prophylaxie est décrit, il consiste en:

1. Adoption de la position latérale, le côté affecté étant le plus élevé;
2. Exercices respiratoires (respiration abdominale) y compris, dans certains cas, la respiration électro-phrénique.
3. Fonctions pleurales fréquentes et traitement avec des agents antimicrobiens spécifiques.

Ces procédés ont été utilisés chez 65 malades. Dans 48 cas, l'épanchement était récent; sur ce nombre, 40 ont présenté une mobilité de ventilation bonne ou très bonne au bout d'une période d'observation de trois mois. Chez 17 malades présentant des épanchements, dont la durée dépassait trois mois au début du traitement, les résultats furent moins satisfaisants. Au bout de cette même période d'observation, 7 malades ont présenté une mobilité diaphragmatique satisfaisante, mais la fonction était considérablement altérée dans 10 cas.

Chez quelques uns de ces malades cependant, une amélioration a été observée au about d'une nouvelle période d'observation de trois mois.

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ANATOMIC RELATIONSHIPS BETWEEN BRONCHOGENIC CARCINOMA AND CALCIFIED NODULES IN THE LUNG[1]

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INTRODUCTION

It is now generally accepted that there has been a striking increase in the incidence of bronchogenic carcinoma throughout the United States (1, 2), although agreement concerning the cause or causes of this increase has not been reached. In several recent papers (3–6) a close association has been noted between bronchogenic carcinoma and calcified foci in the lungs or tracheobronchial lymph nodes in small series of patients. The present study was conceived with the idea of determining in a larger series of cases whether or not bronchogenic carcinoma originates more frequently in those portions of the lung marked by calcified foci than in lung areas not so marked.

An obvious difficulty which has to be faced in making such a study is that calcified foci are an almost universal finding in the lungs of adults in the older age groups. In an attempt to cope with this difficulty, any association between the tumor and the calcified focus was considered to be significant only if the focus was 4 mm. or more in diameter, if it was found in the same lobe as the tumor or in the tracheobronchial nodes draining that lobe, and if it was larger than any calcified focus found in the opposite lung. Using these criteria, clinical records and roentgenograms have been reviewed from cases of bronchogenic carcinoma treated at Herman Kiefer Hospital and at Detroit Receiving Hospital during the past ten years.

MATERIAL AND METHODS

A total of 95 cases was found in the review of the Herman Kiefer Hospital material; all were verified histologically, using tissue obtained from surgically removed lobes, from bronchial biopsy, or at the post-mortem table. In addition to these 95 cases, 21 autopsied cases from Detroit Receiving Hospital were studied.

The frequent difficulty of being certain of the presence or absence of calcified foci in the chest, particularly if only posterior-anterior roentgenograms can be examined, is well known. To partially overcome this difficulty, numerous films from the same case were examined, as well as lateral films when available. In 8 cases, post-mortem films of the removed lungs gave adequate assurance of the presence of calcified foci.

Histologic sections from all cases were reviewed, as a result of which the tumors were classified in four groups: (*1*) small-cell type, (*2*) squamous cell, (*3*) adenocarcinoma, and (*4*) large-cell type (7). Among the 116 reviewed cases, there were 49 in which either the available tissue was inadequate for making a histologic diagnosis or the roentgenograms were completely opaque on the side of the tumor. Sixty-seven cases remained which could be classified according to cell type and according to whether or not calcified foci were found significantly associated with the tumor[2].

[1] From the City of Detroit Department of Health, Detroit, Michigan.

[2] Good, Clagett, and Weed (7) have established the fact that a calcified focus within a

OBSERVATIONS

The 116 original cases have been grouped according to age (table 1). This table confirms other published reports (1, 7*c*, 9) which indicate that bronchogenic carcinoma is primarily a disease of the sixth and seventh decades. Only 10 females were included in the series. Sputum from 66 of the Herman Kiefer Hospital patients was studied for the presence or absence of tubercle bacilli by means of smears or cultures; it was found to be positive for *M. tuberculosis* in only 7 cases.

TABLE 1

CASES OF BRONCHOGENIC CARCINOMA TABULATED ACCORDING TO AGE

AGE GROUP	NUMBER OF CASES
20–29	2
30–39	9
40–49	28
50–59	37
60–69	36
70 plus	4
Total	116

TABLE 2

CALCIFIED PULMONARY FOCI AND BRONCHOGENIC CARCINOMA

Histologic Type of Tumor

SQUAMOUS			UNDIFFERENTIATED						ADENOCARCINOMA		
			Large Cell			Small Cell					
Total Number	Associated Calcified Focus		Total Number	Associated Calcified Focus		Total Number	Associated Calcified Focus		Total Number	Associated Calcified Focus	
	Number	Per cent		Number	Per cent		Number	Per cent		Number	Per cent
19	15	79	20	12	60	22	13	59	6	1	17

The 67 cases which could be classified according to cell type and according to whether or not calcified foci were found closely associated with the tumor are shown in table 2. No great difference was noted in the number of cases of squamous cell, large-cell, and small-cell types; the cases of adenocarcinoma were considerably fewer.

As shown in table 1, there were only 2 cases in the 20- to 29-year age group. Both of these were of the small-cell type. The squamous cell tumors, on the other hand, showed a predilection for the older age groups, none being found in

solitary circumscribed pulmonary mass should suggest the diagnosis of granuloma. As will be seen in figures 1 to 6, the calcified foci associated with bronchogenic carcinoma are not included within the tumor.

patients younger than forty years. Also no squamous cell carcinomas were found among the 10 females of the series.

Table 2 indicates that calcified foci were found closely associated with the tumor in 79 per cent of the cases of squamous cell carcinoma, in 60 per cent of the large-cell undifferentiated type, and in 59 per cent of the small-cell type. Only one of the adenocarcinomas showed an associated calcified focus. In figures 1 to 6 may be seen examples of bronchogenic carcinoma with calcified foci either in the same lobe or in the associated tracheobronchial lymph nodes. It will be noted that the calcified foci, instead of actually being incorporated in the new growth, as is the case in granulomas of the lung (8), are at some distance from the tumor.

DISCUSSION

In considering table 2 it should be remembered that most of the present study was made on the basis of ante-mortem roentgenograms; only eight post-mortem films, which revealed more definitive findings in regard to calcium, were available. It is probable that a study based solely on post-mortem films would show an even closer correlation between calcified foci and bronchogenic carcinoma of the squamous and undifferentiated types than is indicated in table 2.

Calcified foci such as those illustrated in figures 1 to 6 usually mark the area of a healed primary tuberculous lesion. Other possible causes of calcified pulmonary foci, particularly histoplasmosis and coccidioidomycosis, must be considered. In the Detroit area, however, any etiologic agent for these foci other than healed tuberculosis would be rare.[3]

It cannot be doubted that bronchogenic carcinoma is closely associated with calcified foci in many cases. What might be the reasons for this close association? Jackson (4) has suggested that the actual irritation of the stone in a node immediately adjacent to a bronchus may be the important factor. In addition to this possibility, there is the extensive scarring of blood and lymph vessels as well as other pulmonary structures which occurs in the healing of a tuberculous lesion. Moreover, many old tuberculous foci, healed as far as active disease is concerned, show the acicular spaces left by crystals of cholesterol, a compound which, as supplied commercially, has been shown to have a carcinogenic effect (10). Last but not least, *bronchiectasis* is a common sequel of primary tuberculosis (11–14) and bronchiectasis, particularly of the saccular variety, has been indicted (6) as a probable factor in the localization of tars from tobacco smoke.

As already mentioned, it is now generally agreed that there has been a marked increase in the incidence of bronchogenic carcinoma in the United States. While no similar agreement has been reached concerning the cause or causes of this increase, pathologists would be unanimous in accepting at least three factors as being involved in the origin of most malignant growths: (*1*) age of the patient,

[3] During the past ten years Detroit has had a tremendous influx from southern states where histoplasmosis is more common. Most of these new residents, however, are Negroes in whom, for some unexplained reason, bronchogenic carcinoma is not often found.

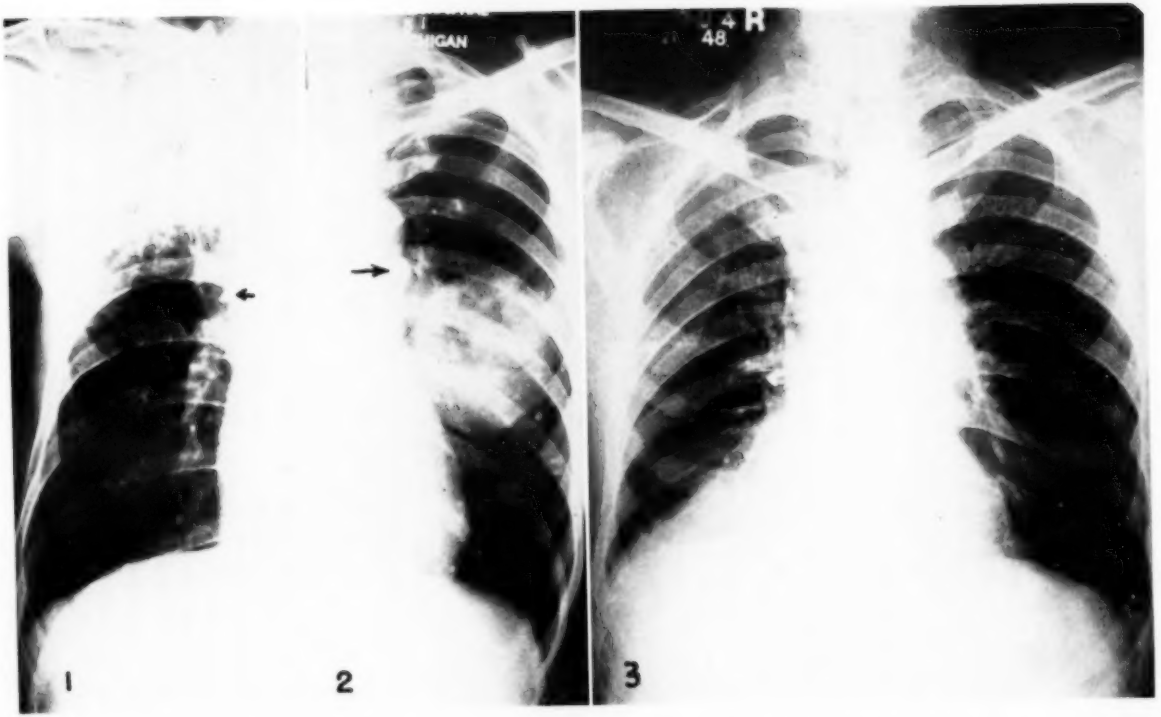

FIG. 1. Bronchogenic carcinoma of right upper lobe. Arrow points to calcified node.
FIG. 2. Bronchogenic carcinoma of left upper lobe. Arrow points to calcified node.
FIG. 3. Bronchogenic carcinoma of right lower lobe

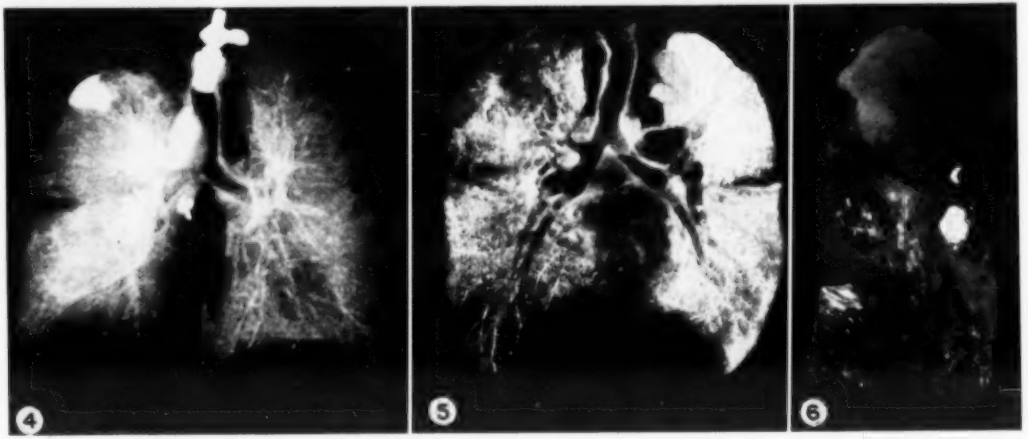

FIG. 4. Post-mortem roentgenogram. Bronchogenic carcinoma of right upper lobe.

FIG. 5. Post-mortem roentgenogram. Bronchogenic carcinoma of left upper lobe. The pulmonary artery has been injected with radiopaque material.

FIG. 6. Post-mortem roentgenogram. Bronchogenic carcinoma involves most of the right upper lobe. Large calcified nodes. The lower lobe contains radiopaque material which was introduced into the bronchus.

(*2*) his familial characteristics or inherited tendency toward the development of cancer, and (*3*) the long-continued action of some type of chronic irritant. During the past few years it has been suggested that tobacco smoke, inhaled into the lungs over a long period of time, is a likely source of the chronic irritant which may cause bronchogenic carcinoma (15–19). One point of evidence cited has been the curves which show that the increased incidence of lung carcinoma in the United States parallels more or less closely the increase in per capita consumption of cigarettes (19). Actually the increased per capita cigarette consumption during the past two or three decades is due largely to the increased use of cigarettes by women. Exclusion of these smokers from consideration would show the curve for per capita cigarette consumption by men rising much more slowly than the curve for incidence of bronchogenic carcinoma. This suggests that another factor, in addition to the smoking of cigarettes, is involved in causing the increased incidence of bronchogenic carcinoma.

It would seem from the present study, as well as from the work of others (4–6), that in many cases the various pulmonary abnormalities left by the healing of a primary tuberculous focus are factors of importance in the localization of bronchogenic carcinoma. Accepting this premise, the lowered death rate from tuberculosis becomes another possible reason for the increased incidence of primary carcinoma of the lung. Thus, prior to 1930, the death rate from pulmonary tuberculosis in young adult males was exceedingly high (table 3). In the 25- to 34-year age group, for example, the range was from 332 per 100,000 in 1890 to 94.5 in 1930 (table 3, figure 7). The striking change in the curves in figure 7 means that a great many males in the first three decades of manhood, who prior to 1930 would have succumbed to tuberculosis, have survived in increasing numbers during more recent years. Instead of being eliminated by death, these persons healed their respective primary tuberculous lesions, with scarred lungs and calcified tracheobronchial lymph nodes as the end result. In other words the decrease in the death rate from pulmonary tuberculosis has been accompanied by a present-day *increase* in the number of persons in the 50- to 60-year age group with healed primary tuberculous foci. This statement is believed to be justified by the following facts:

1. At the present time the incidence of calcified foci in the tracheobronchial lymph nodes of persons 50 years of age and older is virtually 100 per cent (3, 22).

2. The factor of reduced exposure to *M. tuberculosis*, important though it is now, was not important in 1900 when today's 50-year-olds were born. In 1900 the over-all death rate from tuberculosis was still above 200 per 100,000 population and there had not been, as yet, any concerted movement for the isolation of "open" cases of the disease. The exposure to open tuberculosis during infancy and childhood of the groups indicated by the five upper curves in figure 7 (men born from 1860 to 1900) would, it is believed, have been approximately equal.

3. With approximately equal childhood exposure, a fall in the death rate of young males from 332 in 1890 to 95.4 in 1930 should mean that approximately three times as many of the exposed individuals survived to more mature years, for the present writers agree with Rich (23) that by far the most important influence leading to the phenomenal decline in the tuberculosis mortality rate during the first few decades of

this century has been the improvement in living standards and working conditions associated with America's increasing industrialization.

TABLE 3

DEATH RATES FROM PULMONARY TUBERCULOSIS IN MALES ACCORDING TO AGE AT DIFFERENT PERIODS IN THE HISTORY OF THE UNITED STATES

AGE GROUP	YEAR							
	1880*	1890*	1900*	1910*	1920*	1930*	1940†	1949†
15–24	206	212	174	138	98.3	54.2	27.2	10.7
25–34	306	332	280	230	147.0	94.5	52.6	22.0
35–44	299	314	26[illegible]	272	166.0	102.0	70.8	40.4
45–54	300	310	246	247	170.0	113.0	90.9	61.8

* U. S. registration, states of 1900. Data from article by Persis Putnam (20).

† Data for 1930 from 47 states, Texas excluded. Data for 1940 and 1949 from 48 states, deaths among armed forces overseas excluded. Data for 1930, 1940, and 1949 kindly furnished by I. M. Moriyama (21).

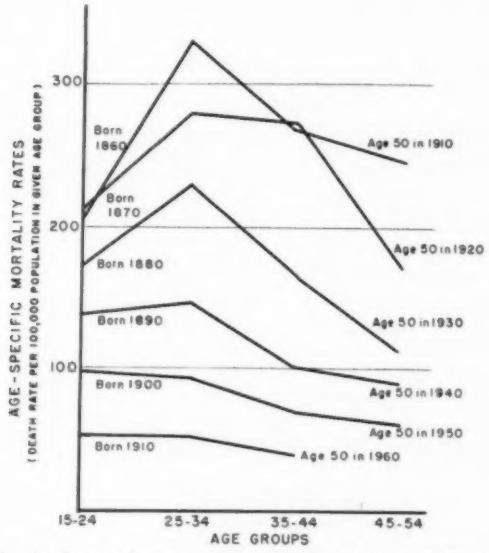

FIG. 7. Death rates from pulmonary tuberculosis in males according to age at different periods in the history of the United States.

Returning now to the problem of carcinoma, it seems evident that the young men who, because of better living standards and working conditions, were able to survive their primary tuberculous infection are now, 30 to 40 years later, the persons particularly susceptible to the development of carcinoma of the

lung. The general increase in life expectancy in the United States has one obvious effect in allowing more persons to reach the "cancer age." That this cannot explain the marked increase in bronchogenic carcinoma is evident from the fact that the death rate from most malignant tumors has been decreasing at the same time that the death rate in the bronchogenic group has been increasing (2).

The concept which has been outlined above gives reason for hope for the future with regard to bronchogenic carcinoma. Were the smoking of cigarettes the leading factor in the causation of lung carcinoma it would be ". . . frightening to speculate on the possible number of bronchogenic cancers that may develop . . ." (19). On the other hand, if healed primary tuberculosis is, as the present writers believe, the more important factor, one would expect the present steep climb in the death rate from bronchogenic carcinoma to continue for only a few years, then level off and begin to decline with the growth to mature years of today's young men who, in their childhood, were not exposed to "open" tuberculosis as their fathers were.

From a more practical point of view, it should be recognized at the present time that a calcified focus in the lungs or tracheobronchial lymph nodes marking an area of healed primary tuberculosis may also mark a pulmonic area particularly susceptible to the development of bronchogenic carcinoma.

SUMMARY

Sixty-seven cases of primary carcinoma of the lung have been reviewed to determine the histologic type and the presence or absence of calcified foci closely associated with the tumor. The majority of the cases of squamous cell and undifferentiated cell types did show associated calcified foci.

The marked drop in the death rate from tuberculosis in young adult males during the past 50 years is suggested as a possible reason for the present noticeable increase in the incidence of bronchogenic carcinoma.

SUMARIO

Relaciones Anatómicas entre el Carcinoma Broncógeno y los Nódulos Calcificados del Pulmón

Sesenta y siete casos de carcinoma primario del pulmón fueron repasados a fin de determinar la forma histológica y la presencia o ausencia de focos calcificados íntimamente asociados con el tumor. La mayoría de los casos escamocelulares y de células no diferenciadas revelaron tal asociación.

Como posible causa del notable aumento actual en la incidencia del carcinoma broncógeno señálase la pronunciada baja de la mortalidad tuberculosa en los varones jóvenes durante los últimos 50 años.

RESUME

Rapports anatomiques entre le cancer bronchogène et les nodules calcifiés du poumon

Des observations de 67 cas de cancer primitif du poumon ont été examinées pour en déterminer le type histologique et la présence ou l'absence de foyers calcifiés, directement associés à la tumeur. La majorité des cas d'épithéliomas du type cylindrique et d'anaplasie présentaient des foyers calcifiés associés.

L'abaissement marqué du taux de mortalité par tuberculose chez le jeune adulte, au

cours des cinquante dernières années, est considéré comme une raison possible de l'accroissement notable observé à présent dans la fréquence des cas de cancer bronchogène.

Acknowledgment

The authors wish to thank Dr. W. L. Brosius for permission to use material from a number of post mortems, including the roentgenograms from which figures 4 and 5 were made; also we wish to thank I. M. Moriyama of the Mortality Analysis Branch of Dr. Dunn's National Office of Vital Statistics.

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CLINICAL AND HISTOPATHOLOGIC STUDY OF THE EFFECT OF CORTISONE AND CORTICOTROPIN ON TUBERCULOSIS[1, 2]

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INTRODUCTION

A number of studies (3–6) in animals have demonstrated that treatment with cortisone[3] has an unfavorable effect upon tuberculosis. Trials of cortisone and corticotropin in human tuberculosis have been relatively few. Furthermore, the studies thus far reported on human patients have not included the histopathology of the tuberculous lesions while under this therapy. The present investigation was carried out in an effort to illustrate the effect of the hormones on the tuberculous lesion by means of biopsies of accessible mucous membrane lesions. It is presumed that the effects on tuberculous tissue should be essentially the same in these lesions as in the lungs.

In human patients, Freeman and associates (1) have described the effects of prolonged therapy with these substances in patients with pulmonary tuberculosis. LeMaistre and co-workers (2), in an excellent study of seven patients with advanced pulmonary tuberculosis, reported the short-term therapy findings. Among these were four patients with tuberculous laryngitis in whom epidermization of laryngeal ulceration was noted within a period of ten days. Sudden withdrawal of the drug was followed by abrupt return of all previous signs of acute illness without return of the laryngeal ulcerations. Such a result is of importance because of the possibility that these powerful short-term effects may in some way be combined with the slower action of the antimicrobial drugs.

METHOD

Six patients with extensive pulmonary tuberculosis and sputum positive for *M. tuberculosis* were studied. All had laryngeal tuberculosis. In addition, one patient had tuberculous lesions of the hard palate with ulceration; and one had an ulcer in the region of a previously removed left tonsil. Although markedly ill, all of the patients were able to walk.

Biopsies and photographs of the lesions were made one to three days before, and on the last day of, treatment.

Eosinophile counts were performed at various times on each patient. The technique used was the propylene glycol method described by Randolph with the following modification: One set of 4 accurately standardized hemocytometer white pipettes was used in all of the counts. Two counts were done in each pipette and the total number of eosinophils in the 9 large millimeter squares of the Levy-Hauser chamber was recorded for each count.

Development of factor for number of eosinophils per cu. mm.: $\frac{20 \times 10}{8 \times 9} = 2.8$ (factor) where 20 corrects for dilution, 10 for depth of fluid on chamber, and 8 and 9 allow for average count and average sq. mm., respectively.

[1] From the Municipal Tuberculosis Sanitarium, Chicago, Illinois.

[2] Presented before the Chicago Tuberculosis Society, October 29, 1951.

[3] Generously supplied by Merck & Co., Rahway, New Jersey.

The clinical condition of the patients was followed by means of studies of the temperature, weight, expectoration, and general condition. The intracutaneous reaction to tuberculin was determined before, during, and after treatment, using 1:1,000,000, 1:100,000, and 1:10,000 Old Tuberculin (OT) simultaneously, followed by stronger concentrations if required. Blood counts, urinalyses, sputum studies, and roentgenograms of the chest were made. Treatment with corticotropin or cortisone was carried out for periods of ten to twenty-two days (*See* table 1 for general data and results).

Observations

Case Reports

Patients Who Received Cortisone

Case 1. W. W., a Negro male, age 40 years, weighed approximately 95 pounds on admission on March 23, 1951. Roentgenograms revealed infiltration in the right upper lobe and involvement with cavitation throughout the left lung. Pain and hoarseness had been noted for several weeks. Laryngeal examination revealed a rounded mass at the posterior commissure. Sputum was positive for *M. tuberculosis*.

Cortisone, 100 mg. daily, was given intramuscularly from May 19, 1951 to June 3, 1951. Eosinopenia occurred.

Examination and photographs of the larynx revealed no change following the treatment. Biopsy findings are described later in this report. No improvement was noted after three weeks of dihydrostreptomycin and para-aminosalicylic acid (PAS). The patient's general condition failed slowly to October, 1951 (figure 1).

Case 2. W. R., a Negro male, age 37 years, weighed 102 pounds on admission on May 21, 1951. A roentgenogram of the chest revealed extensive bilateral infiltration and cavitation. Sputum was positive for *M. tuberculosis*, and his temperature was 101° F. Hoarseness without pain was noted for three months. The larynx revealed great swelling of both arytenoids with superficial ulceration.

Cortisone, 100 mg. daily, was given intramuscularly from May 26, 1951 to June 16, 1951 (twenty-two days).

The eosinophils diminished from 27 per cu. mm. to 10 per cu. mm. The patient's weight increased to 113.5 pounds and his fever diminished.

At the end of therapy he was still hoarse, but said he felt better. Examination and photographs, however, revealed considerable progression of disease. There was an increase in the swelling, especially of the epiglottis, with marked infiltration of both arytenoids and aryepiglottic folds. Ulceration was still present. The biopsy findings are described later in this report.

Marked improvement followed treatment with dihydrostreptomycin and para-aminosalicylic acid (PAS) (started June 16, 1951). The patient's voice returned in one week.

Case 3. F. A., a Mexican male, age 29 years, weighed 107 pounds on admission on July 26, 1951 and had irregular fever to 101° F. A chest roentgenogram revealed infiltration and cavitation in the upper half of the lungs bilaterally. The sputum was positive for *M. tuberculosis*. Hoarseness without pain was present for six months.

Cortisone, 100 mg. daily, was injected from August 10, 1951, to August 15, 1951. Eosinophils declined from 142 per cu. mm. to 110 per cu. mm. The dose was increased to 200 mg. daily until August 25, 1951. The eosinophils diminished to 26 per cu. mm. The weight increased to 132 pounds at the end of therapy, and fever diminished. Mild euphoria occurred.

Before treatment, examination of the larynx revealed extensive disease of the lateral walls, especially on the left. No change was noted at the end of therapy.

TABLE 1

DATA ON SIX PATIENTS TREATED WITH CORTISONE OR CORTICOTROPIN FOR TEN TO TWENTY-TWO DAYS

CASE NUMBER	RACE, SEX, AND AGE	WEIGHT	DAILY TREATMENT	DURATION OF TREATMENT	EOSINOPHILS PER CU. MM.		EFFECT OF TREATMENT ON HISTOPATHOLOGY	GROSS APPEARANCE OF MUCOUS MEMBRANE LESION AFTER TREATMENT
					Before Treatment	After Treatment		
		pounds		*days*				
1	W. W., Negro male, 40 years	95	Cortisone, 100 mg. intramuscularly	14	?	3	Increased inflammatory reaction; less limitation of tubercles after treatment; no tubercle bacilli found before treatment; tubercle bacilli present after treatment	No change
2	W. R., Negro male, 37 years	102	Cortisone, 100 mg. intramuscularly	22	27	10		Worse: more swelling
3	F. A., Mexican male, 29 years	107	Cortisone, 100 mg. 200 mg.	5 10	142	110 26		No change
4	W. M. B., Negro female, 34 years	127	Corticotropin, 20 mg. intravenous drip	10	343	33	Inflammatory reaction slightly less after treatment; tubercle bacilli present before treatment (insufficient specimen for post-treatment examination for bacilli)	No significant change
5	L. K., white male, 33 years	106	Corticotropin, intravenous drip	12	153	25	Tubercules atypical after treatment, with less giant cells; more tubercle bacilli after treatment	No change
6	N. E., white male, 51 years	103	Corticotropin, 50 mg. intramuscularly	10	102	29	Increased inflammatory reaction; more tubercle bacilli after than before treatment	No change

Dihydrostreptomycin and PAS were started on August 27, 1951 and were followed by marked improvement in laryngeal symptoms. Weight increased to 137 pounds by September 17, 1951.

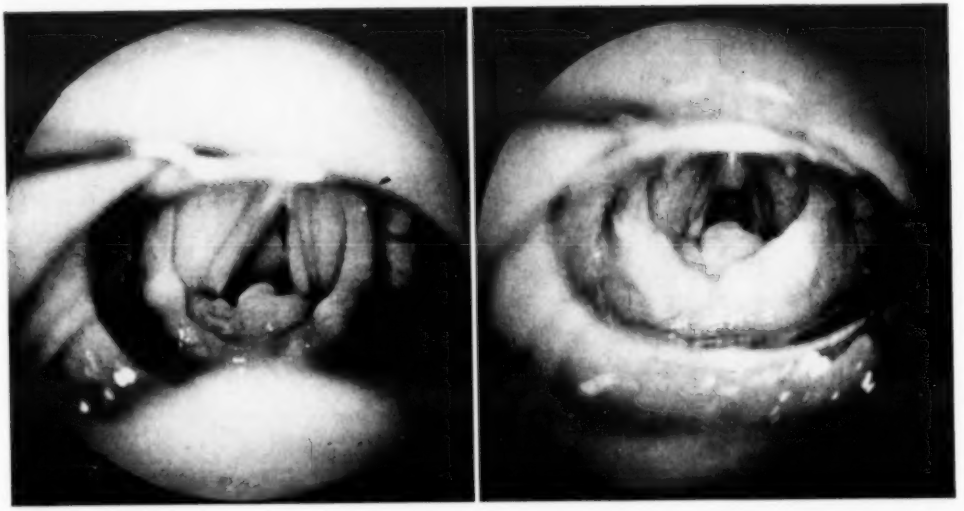

FIG. 1A. Photograph of the larynx of patient W. W. (*Case 1*), a 40-year-old Negro male with far advanced bilateral pulmonary tuberculosis; he complained of hoarseness and pain in the throat for one month. Note the large tuberculoma of the posterior commissure.

FIG. 1B. Appearance after the administration of 100 mg. of cortisone intramuscularly for fifteen days. The tuberculoma is unchanged, but there is increased edema of the posterior portion of the larynx.

Patients Who Received Corticotropin

Case 4. W. M. B., a Negro female, age 34 years, was admitted on October 4, 1951. She dated the onset of her symptoms to April, 1951. A chest roentgenogram revealed dense consolidation of the right upper lobe with a cavity four centimeters in diameter. The sputum was positive for *M. tuberculosis*. She complained of hoarseness and severe pain in the left side of the throat for three weeks. Laryngeal examination revealed infiltration and hyperemia of both cords with superficial ulceration. A deep ulcer was noted over the left vocal process. Corticotropin, 20 mg., was given by intravenous drip over an eight-hour period daily from October 11, 1951 to October 20, 1951, inclusive. The eosinophile count was 343 per cu. mm. before treatment and varied from 33 to 151 per cu. mm. during treatment. Her weight increased from 125 to 133 pounds. At the end of treatment the patient thought her voice was better but still complained of pain in the throat. Laryngeal examination and photographs revealed hyperemia of both vocal cords. The superficial ulceration was not noted. The ulcer of the left vocal process was still present, though possibly smaller.

Tuberculin tests revealed a marked depression of hypersensitivity during treatment. Reactions examined five days after the end of treatment had returned to their pretreatment state.

Roentgenograms after therapy revealed the most marked clearing noted in any of the cases in this series (figure 2). Considerable resorption of the right upper lobe density occurred.

Case 5. L. K., a 33-year-old white male, was admitted on November 2, 1951; he weighed 106 pounds. The onset of symptoms in March, 1950 was catarrhal. He was ambulatory but weak and dyspneic on admission. Roentgenograms revealed bilateral mottled infiltration of the lungs with cavitation. Sputum was positive for *M. tuberculosis*. The patient complained of pain in the throat, without hoarseness, for six months. Examination on November 10, 1951 revealed an ulcer occupying the left tonsillar fossa, including the anterior and posterior pillars. (The patient had had a tonsillectomy in 1927.) A large superficial ulcer was also present which involved the entire right lateral wall of the larynx, base of the epiglottis, and right false cord. The true cords were not involved.

Corticotropin was started on November 13, 1951 by intravenous drip for approximately eight hours daily (20 mg. per day) until November 24, 1951 (12 days). Potassium chloride, 1 gm. four times daily, was given, and the patient received a low salt diet. On November 17, 1951, after three days of treatment, the patient stated that he had no more pain in the throat. Examination revealed no change in the appearance of the ulcers on November 17, 1951, nor on November 24, 1951 after twelve days of the above therapy.

The eosinophile count before treatment was 153 per cu. mm. During treatment they varied from 25 to 82 per cu. mm.

During treatment the patient was afebrile from November 13, 1951 to November 17, 1951 and gained four pounds; then he developed fever ranging from 101° to 104° F. daily, with loss of seven pounds during the subsequent nine days. Fever persisted in spite of 600,000 units of penicillin daily by intramuscular injection, but fell to 99.2° F. in four days after corticotropin treatment was stopped.

Roentgenograms of the chest revealed no change at the end of therapy with corticotropin. The tuberculin reaction during treatment revealed only slight depression of reaction.

Biopsy of the tonsillar fossa lesion before corticotropin revealed many typical tubercles with numerous giant cells, some tubercle bacilli, and ulceration of the epithelium. At the end of therapy, the tubercles were atypical in appearance, with only a rare giant cell but with more tubercle bacilli. Ulceration was still present.

Case 6. N. E., a white male, age 51 years, weighed 103 pounds on admission on April 16, 1951. He had bilateral far advanced pulmonary tuberculosis with cavitation. Ulceration of hard palate and involvement of the epiglottis were present. The sputum was positive for

M. tuberculosis. The patient had hoarseness and pain on swallowing for three and one-half months before admission.

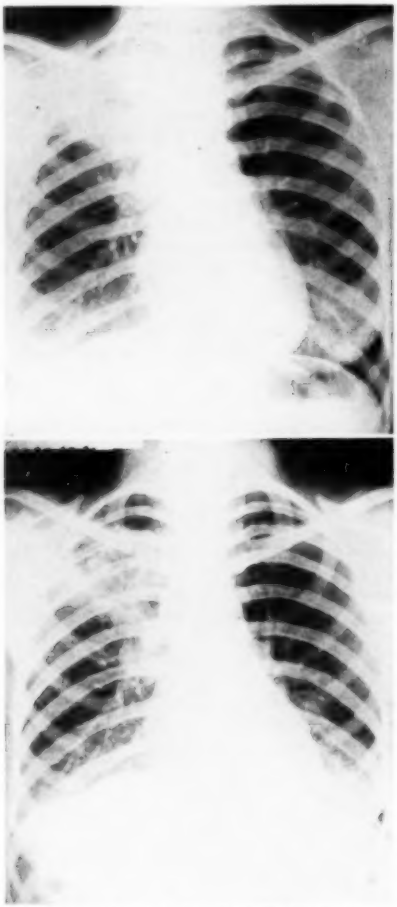

FIG. 2. (Top) Roentgenogram of chest of patient W. M. B. (*Case 4*) taken on October 4, 1951, before treatment. Density in right upper lung field with large excavation. (Bottom) Roentgenogram taken on October 22, 1951, after ten days of corticotropin (20 mg. daily by intravenous drip). Marked clearing of density.

Corticotropin, 50 mg. daily by the intramuscular route, was given for ten days beginning on April 26, 1951. The eosinophile count was 102 per cu. mm. before treatment and 29 per cu. mm. during treatment. Mild euphoria occurred, but there was no objective improvement in voice or appearance of lesions. Photographs (figure 3) and biopsies (figures 4*A*–*D*)

FIG. 3A. Tuberculous ulceration of the hard palate of patient N. E. (*Case 6*), a 51-year-old male with far advanced bilateral pulmonary tuberculosis. He suffered severe pain and dysphagia.

FIG. 3B. Appearance after treatment with 50 mg. of corticotropin intramuscularly for ten days. The ulceration seems deeper, more evident. The patient stated that he felt better and had less pain.

FIG. 3C. Appearance after seven weeks combined therapy with dihydrostreptomycin and PAS. The ulceration has entirely healed; only slight irregularity of the mucosa remains; complete absence of pain.

FIG. 4A. Pretreatment biopsy from patient N. E. (*Case 6*), showing that the epithelium is intact and that there is a fairly well-preserved stroma. The most prominent feature is the marked cellular infiltration in the lower left-hand corner. (× 30) Hematoxylin and eosin stain

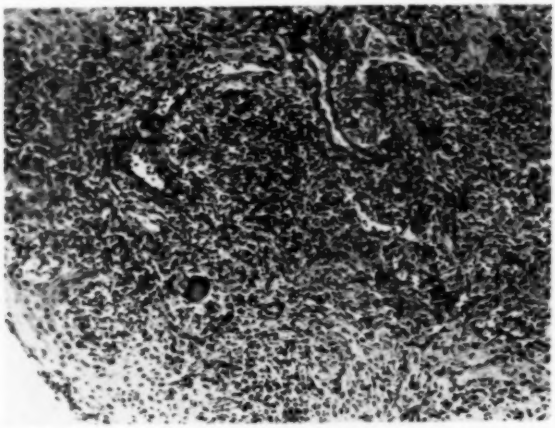

FIG. 4B. High-power view of most involved area of figure 4A showing a giant cell, epithelioid cells, and many chronic inflammatory cells. Note that the vessels are intact and no caseation necrosis is apparent. (× 100) Hematoxylin and eosin stain

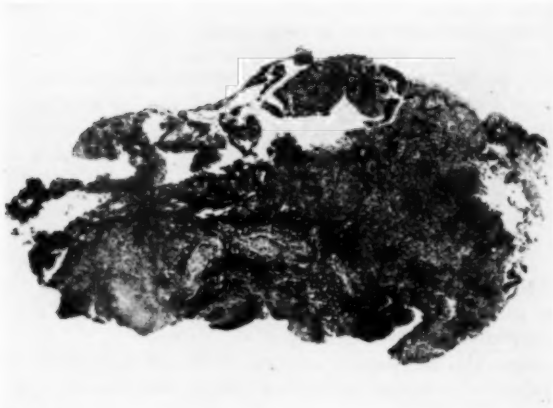

FIG. 4C. Post-treatment biopsy from patient N. E. (*Case 6*) demonstrating denudation of almost all of the epithelium and a massive infiltration of cells, with destruction of the stroma and some caseation necrosis. (× 30) Hematoxylin and eosin stain

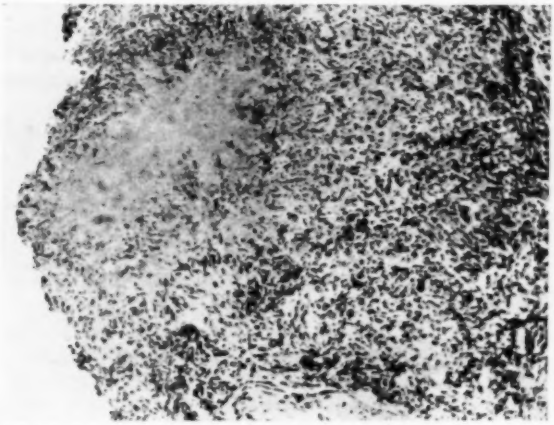

FIG. 4D. High-power of figure 4C showing area of caseation necrosis, many chronic inflammatory cells, but few epithelioid cells and no giant cells

of the lesions before and after corticotropin show no evidence of improvement. Treatment with dihydrostreptomycin and PAS was started on May 8, 1951 and was followed by marked subjective and objective improvement in twelve days.

Subjectively the patients felt better and presented evidence of mild euphoria during therapy. Cough diminished and most of the patients gained weight. Fever was absent during therapy in Cases 1, 2, 3, 4, and 6, except for an occasional slight rise. Fever recurred when therapy was discontinued and was exceptionally high (102° to 104°F.) for a few days in Cases 1 and 6. Case 5 had irregular fever during therapy, as described above.

Although some of the patients thought their voices were improving, the observers were unable to note any definite change. No improvement could be seen in weekly inspections of the laryngeal and palatal lesions during treatment. In one patient the laryngeal lesion appeared worse after therapy. Color photographs corroborated these findings.

Histopathology: The microscopic changes in biopsy material of the mucous membrane lesions were studied in 6 cases treated with corticotropin and cortisone. This evaluation was made by the use of sections stained in the conventional manner with hematoxylin and eosin, sections stained for connective tissue (Masson's trichrome stain), and sections stained especially for acid-fast bacilli (Ziehl-Neelsen). The diagnosis of tuberculous laryngitis in pretreatment biopsies was based on the presence of typical discrete tubercles showing epithelioid cells, varying numbers of giant cells of the Langhans' type, peripheral lymphocytes, and some degree of caseation-necrosis. Case 4 failed to display any discrete tubercles in either the pretreatment or the post-treatment biopsies, but the finding of many acid-fast bacilli confirmed the diagnosis.

In the 6 cases studied, the tubercles were found to be limited to the stroma, in most instances quite removed from the subepithelium. Some changes were noted in the epithelial layer, which could be considered common to any type of inflammatory process. However, 3 patients demonstrated some ulceration prior to therapy, and 2 patients demonstrated some ulceration following therapy.

The outstanding differences between the pre- and post-treatment biopsies were as follows:

The inflammatory reaction in the submucosal structures was increased following therapy with corticotropin and cortisone. This reaction was characterized by the presence of ill-defined tubercles showing varying amounts of caseation, few to many giant cells, large numbers of monocytes, plasma cells, lymphocytes, and an occasional polymorphonuclear leukocyte. In contradistinction to the discrete well-delimited tubercles seen before therapy, the post-treatment biopsies revealed a more diffuse type of inflammation.

The presence of acid-fast bacilli in all of the biopsies following therapy contrasted with the finding of acid-fast bacilli in only two biopsies prior to therapy. (Case 4 was excluded because insufficient tissue was present in the post-treatment biopsy to warrant comparison.) While this is a qualitative measurement only and liable to considerable variation, it is believed to be of great significance in view of the fact that many sections were examined. From this observation one would have to conclude that the tubercle bacilli

were present in greater numbers following the use of the two drugs than they were before therapy was instituted.

Sections stained with Masson trichrome stain did not reveal any appreciable degree of fibrous tissue proliferation at the end of therapy. The formation of scar tissue was hardly to be expected in the short time which elapsed between biopsies.

Roentgenograms: The serial roentgenograms of the lungs showed marked clearing of the lesion at the end of treatment in Case 4 (figure 2). Partial clearing was observed in Case 3. The other 4 patients showed no significant changes.

Tuberculin reaction: Marked depression of reactivity to tuberculin occurred during treatment in 2 patients, moderate depression in one, and little or no change in the other 3 patients. Reactivity returned to the pretreatment state in five days in one case and within a few weeks in all. Measurements are shown below for Cases 3 and 4.

INTRACUTANEOUS TUBERCULIN TESTS

F. A. CASE 3	OT		
	1:10,000	1:1,000	1:100
July 20, 1951, before treatment (cortisone)	0.3 cm.	0.8 cm.	
August 17, 1951, during treatment	0.3 cm.	0.6 cm.	0.8 cm.
October 8, 1951, after treatment	0.5 cm.	1.0 cm.	1.5 cm.
W. M. B. CASE 4	OT		
	1:1,000,000	1:100,000	1:10,000
October 8, 1951, before treatment (corticotropin)	0.5 cm.	0.8 cm.	1.2 cm.
October 15, 1951, during treatment	0.1 cm.	0.4 cm.	1.0 cm.
October 22, 1951, after treatment	0.5 cm.	1.0 cm.	1.2 cm.
November 5, 1951, after treatment	0.1 cm.	0.8 cm.	1.3 cm.

Eosinophile counts: Eosinophile counts were performed just prior to injection of the hormone and at intervals of two, four, or six hours afterward. Additional details concerning the eosinophile counts in Cases 3, 4, and 5 on various days during the period of treatment are given in table 2.

DISCUSSION

The effect of cortisone on the pathology of tuberculosis has been described in animals (3–6) as a diminution of capillary permeability and a decrease in the inflammatory response. In spite of this apparent improvement in the appearance of the lesion, an increase in the growth of tubercle bacilli occurs mostly within the phagocytic cells. The long-range results in animals have indicated that unfavorable progression of the disease takes place with an ultimately harmful effect.

In human patients with pulmonary tuberculosis it has been reported that cortisone and corticotropin cause progression of the disease although there may be improvement in symptoms. In general, the findings in the present study are in agreement with those of Freeman and associates (1) and of LeMaistre

and co-workers (2) except in regard to the effects on laryngeal lesions. The findings reported herein concerning mucous membrane lesions are directly contrary to the previous findings. Photographs and biopsies made before and after therapy indicate that no significant improvement occurred in the cases in the present study.

Two possible sources of error need to be considered. The first of these is the small number of patients treated. The possibility cannot be excluded that this therapy may act differently on other patients.

TABLE 2

EOSINOPHILE COUNTS IN PATIENTS RECEIVING HORMONES

CASE	DATE	EOSINOPHILS PER CU. MM.		PER CENT CHANGE	COMMENT
		Before Injection	After Injection		
A. Cortisone					
F. A. Case 3	8–10–51	142	122	14% decrease in 4 hours	100 mg. intramuscularly daily
	8–13–51	124	110	12% decrease in 4 hours	100 mg. intramuscularly daily
	8–17–51	22	26	27% increase in 4 hours	After 200 mg. intramuscularly daily
B. Corticotropin					
W. M. B. Case 4	10–11–51	343	114	66% decrease in 4 hours	First day of treatment
	10–15–51	63	151		2 hours after starting infusion
			33	48% decrease in 6 hours	6 hours after starting infusion
	10–17–51	100	103		2 hours after starting infusion
			33	67% decrease in 6 hours	6 hours after starting infusion
L. K. Case 5	11–13–51	153	82	46% decrease in 4 hours	First day of treatment
	11–16–51	77	74		2 hours after starting infusion
			25	68% decrease in 6 hours	6 hours after starting infusion
	11–19–51	148	29	79% decrease in 4 hours	4 hours after starting infusion
	11–21–51	163	81		2 hours after starting infusion
			33	81% decrease in 6 hours	6 hours after starting infusion

The second possible error is inadequate dosage. The dosage used in this series is believed to be adequate for the following reasons: The patients were underweight; the usual eosinopenic response was obtained; all the patients noted mild euphoria; and gain in weight occurred. One patient gained 18 pounds in fifteen days. It is believed, therefore, that failure of therapeutic effectiveness cannot be attributed to inadequate dosage in these patients.

The increase in the number of tubercle bacilli noted in the tissues after treatment is consistent with the pathologic effects previously reported in animals. The increase in the inflammatory response seen in the biopsies of the larynx differs from the effects noted in animal experiments. This perhaps may be due

to the differences between the acute type of tuberculosis usually seen in animals and the more chronic infection in the human.

It is difficult to account for the partial clearing of roentgenographic evidence of pulmonary disease noted both in these cases and by other observers. It is hardly credible that the hormones may affect the disease differently in the lung and in the larynx. A possible explanation may be as follows: A large component of the pulmonary lesion is a hypersensitive reaction, due to the presence of relatively enormous numbers of tubercle bacilli. This reaction is markedly diminished by the hormones. In the larynx, however, there is little hypersensitive response, due to the paucity of tubercle bacilli (as noted in the pretreatment biopsies) and, therefore, less effect from the hormones.

It may also be possible that some part of the laryngeal pathology is nonspecific rather than tuberculous and may, therefore, respond differently to the hormones.

It well may be that the roentgenographic clearing depends mainly upon resorption of edema. This possibility coincides with the fact that the clearing is more marked in the more recent or "exudative" pulmonary lesions than in the "productive." Biopsies of the laryngeal lesions show relatively little edema and present much less possibility of great volume of resorption because of the solid nature of the tissue as compared to the spongy texture of pulmonary tissue.

The subsequent improvement under streptomycin therapy demonstrates clearly that the patients were capable of responding to appropriate medication.

It should be mentioned in passing that there has been much speculation concerning the possibility of combining cortisone or corticotropin with streptomycin in the treatment of pulmonary tuberculosis. Thus far, no benefit has been reported from attempts in this direction. No definite clinical harm was observed in the patients in the present study as a result of the short courses of cortisone and corticotropin, nor was any difference in the effects produced by the two substances noted.

Summary

Six patients with advanced pulmonary tuberculosis and tuberculous laryngitis were treated with cortisone or corticotropin for periods of ten to twenty-two days. In addition to the usual studies, photographs and biopsies of the larynx were made before and after therapy. The photographs revealed no definite improvement in the gross pathology. Examination of biopsy material at the end of treatment revealed increased inflammatory response, lessened limitation of tubercles, and increased numbers of tubercle bacilli. Roentgenograms of the chest showed definite clearing of the lesions in 2 patients. Tuberculin sensitivity was depressed during therapy.

Sumario

Estudio Clínico e Histopatológico del Efecto de la Cortisona y la HACT sobre la Tuberculosis

Seis enfermos con tuberculosis pulmonar avanzada y laringitis tuberculosa fueron tratados con cortisona o HACT durante períodos de tiempo que variaron de diez a veintidós

días. Además de los habituales estudios, se tomaron cromofotografías y biopsias de la laringe antes y después de la terapéutica. Las fotografías no revelaron mejoría bien definida en la patología macroscópica. Las biopsias hechas al final del tratamiento mostraron mayor respuesta inflamatoria, menos limitación de los tubérculos y aumento en el número de bacilos tuberculosos. Las radiografías torácicas revelaron despejo bien definido de las lesiones en 2 enfermos. La sensibilidad a la tuberculina descendió durante la terapéutica.

Resume

Etude clinique et histo-pathologique de l'effet de la cortisone et de l'ACTH sur la tuberculose

Six malades, atteints de tuberculose pulmonaire avancée et de tuberculose laryngée, ont été traités par la cortisone ou l'ACTH pendant des périodes de dix à vingt-deux jours. En dehors des études habituelles, des photographies en couleurs et des biopsies du larynx ont été faites avant et après le traitement. Les photographies n'ont pas montré d'amélioration définie des manifestations pathologiques macroscopiques. Les biopsies, pratiquées à la fin du traitement, ont révélé une augmentation de la réaction inflammatoire, l'extension des tubercules et une augmentation du nombre de bacilles tuberculaux. Des radiogrammes du poumon ont montré une nette diminution des opacités chez 2 malades. La sensibilité à la tuberculine a diminué au cours du traitement.

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USE OF RABBIT EYE AS A TISSUE TO STUDY TUBERCULOSIS[1, 2]

III. Effect of Adrenal Hormones upon Course of Disease and upon Certain Immunologic Responses

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With the technical assistance of LEONARD CANARILI

(Received for publication January 29, 1952)

INTRODUCTION

The present report consists of observations on the effect of adrenal hormones (corticotropin and cortisone) upon the course of early and late ocular tuberculosis in the rabbit. In previous communications, the course of tuberculosis in the rabbit's eye, both untreated and treated with antituberculous agents, has been described (1, 2). Evolution of the natural disease and its alterations by streptomycin are sufficiently predictable so that comparison with disease influenced by adrenal hormones is permissible.

METHODS AND MATERIALS

The methods utilized in the present study were similar to those described previously (1, 2). Adult, mongrel, white rabbits, averaging 3 kg. in weight, were infected by direct inoculation into the anterior eye chamber with approximately one million viable human-strain tubercle bacilli (H37Rv). This represents a large-sized inoculum and in the untreated animal results in advanced but localized ocular disease in 75 per cent of the animals.

The course of optic tuberculosis in 39 rabbits was observed. Twelve animals served as controls and 27 were treated with one or the other hormone. None of the rabbits was immunized prior to therapy although the cutaneous reaction to tuberculin was positive in 18 animals when hormone administration was initiated. Sensitization to Old Tuberculin (OT) occurred in 5 of these as a result of previously established eye disease and, in the remaining animals, sensitization presumably resulted from frequent skin testing with 50 per cent OT prior to ocular infection (designed for a separate study upon the Middlebrook-Dubos hemagglutination test (3)).

The dosage of corticotropin was 1 mg. per kg. of body weight per day divided in three equal doses administered subcutaneously every eight hours. Two and five-tenths to 10 mg. of cortisone[3] were administered parenterally once daily. Corticotropin was continued for five to fourteen days and cortisone was given for fourteen days.

Vitamin C determinations were made upon the adrenal glands by the method of Roe and Kuether (4). The glands were weighed and fixed immediately after death from air embolism. The vitamin C content was measured within two days of fixing in 5 per cent metaphosphoric acid and 10 per cent acetic acid solution[4].

Hemagglutination tests were performed by a modification of the Middlebrook-Dubos

[1] From the Department of Medicine, State University of New York, College of Medicine, Syracuse, New York.

[2] This study was aided by a grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association.

[3] Supplied by Merck & Co., Rahway, New Jersey.

[4] Through the courtesy of Dr. Jay Tepperman, Department of Pharmacology, State University of New York, College of Medicine, Syracuse, New York.

method (3, 5). Concentrated fraction 1A of Old Tuberculin used in the test was of standard source and potency[5], and sheep erythrocytes were obtained from a single animal.

Old Tuberculin, 25 or 50 per cent, was injected subcutaneously in the flank. The infecting strain of *M. tuberculosis* var. *hominis* was of established virulence for guinea pigs, was susceptible to streptomycin *in vitro*, and had been maintained in Tween[6]-albumin medium.

RESULTS

Corticotropin altered appreciably the course of ocular tuberculosis in the rabbit when administered prior to, at the time of, or shortly after optic infection was induced. The type and degree of alteration varied with timing of the first dose of the hormone. Cortisone in the dosage used did not produce as great a change. Neither hormone had a significant local effect upon old, established ocular disease, but systemic complications were noted in the treated animals with aged eye disease.

Corticotropin administered at the time of, or shortly after, ocular infection: Eight rabbits were given corticotropin within five to fourteen days after initiation of ocular tuberculosis. Corticotropin was started in 2 other rabbits within twenty-four hours of optic infection. During the pretreatment period, after inoculation of the eyes, the infection had become evident in twenty-four hours, had progressed to the described minimal stage in five days, and had become moderately advanced in two weeks (1). With institution of hormonal therapy, the expected course of ocular disease was observed to change and to progress in a rapid, fulminating, and unexpected manner in all of the 10 animals. The impressive speeding of the infection to the totally destroyed eye was noticeable uniformly within twenty-four hours after the first dose of the hormone. Within seven days after the first dose of the hormone, all eyes were ravaged and thereafter became totally destroyed, with no evidence of organization, revascularization, or localization of the infection. Empyema of the anterior chamber was evident, with bulging of the cornea, but the cornea never ruptured, nor did sloughing occur. At termination of the treatment period, no possible further damage save complete sloughing could have occurred in that part of the infected eye which was grossly visible to the observers.

In figures 1 *A*, *B*, and *C* may be seen the course of the disease in one of the animals whose therapy began within twenty-four hours of ocular infection. It is typical of the changes observed in the other 9 animals. The fulminating course of the disease is apparent. Figure 1*A* represents the appearance of the process after four days of treatment; figure 1*B*, at the end of fourteen days of therapy; and figure 1*C*, at the time of autopsy, four weeks after institution of eye disease and two weeks after the end of therapy.

The eyes of 3 animals were used as controls, each having been infected with the same inoculum of organism on the same day and from the same medium as

[5] Supplied by H. D. Piersma, Lederle Laboratories, Pearl River, New York.

[6] Tween 80 is the proprietary name of the polyoxyethylene derivative of Sorbitan monooleate, obtained from the Atlas Powder Co., Wilmington, Delaware.

the treated group. Ocular tuberculosis in one of these at the end of the four weeks' observation time was moderately advanced, with mild diffuse iritis and a well-localized tubercle centrally placed and anterior to the lens. Eye disease in the remaining 2 had reached an advanced stage during the month of observation. Tuberculous involvement of iris and lens developed and the anterior chamber gradually became filled with caseous material, but glaucoma and

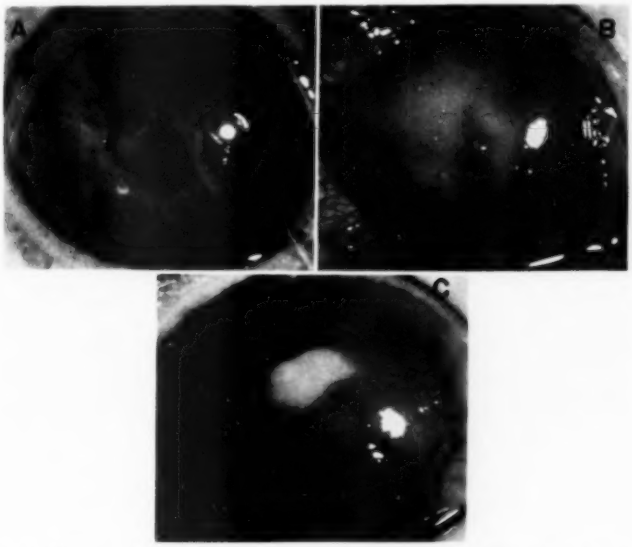

FIG. 1 *A*. Photograph of rabbit eye (A-12) dated February 16, 1951, taken after start of corticotropin and five days after ocular inoculation of tubercle bacilli into anterior chamber. There is an acute, moderately advanced lesion with central tubercle and acute iritis.

B. Photograph taken eleven days later (February 27, 1951) of the eye at end of treatment with corticotropin. Empyema of anterior chamber and glaucoma with destruction of iris are shown.

C. Photograph taken ten days after "*B*" (March 9, 1951) shows continuation of the process to complete destruction of all recognizable architecture of the eye. Figures 5*A*, *B*, and *C* are photomicrographs of this eye. Animal was killed twenty-four hours after this photograph was taken.

empyema within the chamber were never observed. Revascularization from the limbus was prominent after the third week. The difference between the two series, treated and untreated, was striking. Disease in the former group was characterized by acute inflammation and rapid destruction of all parts of the anterior chamber. The controls showed the expected slower replacement of structures with tuberculous granulation tissue, without significant rubor or edema.

During the first two-week post-treatment observation period, the eye lesions failed to show objective evidence of healing.

Throughout the treatment period and for two weeks thereafter, physical health of the 10 animals quickly deteriorated from normal. All lost appetite and weight, they were unusually irritable, their skin lost its sheen, and they appeared generally ill. No animal, however, died during the period of study; each was sacrificed as planned.

Corticotropin administered twenty-four hours prior to ocular infection: In contrast to the effect noted in the above group of animals, corticotropin exerted an initial suppressive effect upon the course of ocular tuberculosis when given prior to institution of the infection. The first three doses of corticotropin were given prophylactically to 3 rabbits. During the seven days of its administration, no gross ocular tuberculosis developed. The needle tract wound, through which the infection was established, did not become inflamed and only a feathery conjunctival cloud kept the eyes from being described as totally normal. Figure 2*A* shows the eye of one of these rabbits six days after ocular infection and seven days' treatment with corticotropin.

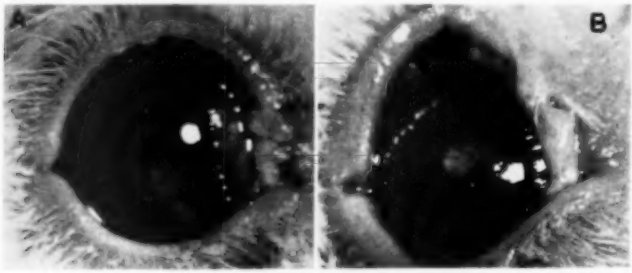

FIG. 2 *A*. Photograph of rabbit eye (A-1) dated February 16, 1951, seven days after start of corticotropin and six days after ocular inoculation of tubercle bacilli. Feathery lesion on conjunctiva is shown. There is also minimal iritis.

B. Photograph taken eleven days later (February 27, 1951) of the eye ten days after the end of treatment with corticotropin. Acute iritis with a moderately advanced tuberculous lesion in the anterior chamber is seen.

While ocular disease was being held in abeyance by the hormone, anterior chamber fluid, although normal in gross appearance, contained culturable tubercle bacilli. The general health of the animals did not change during this first week of infection and treatment.

Within two days of cessation of corticotropin, ocular disease suddenly became apparent and progressed to total destruction of the eye during the next ten days. This occurred in the eyes of all 3 animals. At the time of autopsy, nine to thirty days later, all normal optic architecture was gone, the anterior chamber was filled with viscous, gray pus, and no vascularization was apparent. The course of ocular infection immediately post therapy in this group was sub-

stantially similar to that observed in animals whose eye disease was initiated immediately preceding therapy with corticotropin, and the animals became ill in a like manner. Figure 2*B* represents the appearance of the eye of the same animal ten days after cessation of corticotropin and eleven days after figure 2*A*.

Eye disease in 2 control animals proceeded in a predicted manner. At the end of the first week's observation, lesions in both were at a minimal stage, and slow progression to a more advanced disease took place thereafter. Despite the large-sized inoculum used in the control animals, far advanced lesions did not develop although tuberculous granulation tissue replaced a portion of each eye.

Corticotropin administered six to eight months after establishment of ocular disease: Advanced ocular tuberculosis was allowed to develop in 8 rabbits over a period of six to eight months. From inception to time of administration of corticotropin, the disease in each had become stabilized at an advanced stage and the eye had been replaced by a solid mass of yellow, organized, fibrotic, and caseous material which occupied the entire anterior chamber. No change in the character of the disease had been observed for at least one month prior to institution of treatment. Corticotropin was administered to 5 of these animals; the other 3 were controls. Administration of the hormone for fourteen days failed to alter the ocular disease in any way; at the termination of the therapy period, eyes in the treated group were identical with those of the controls.

Despite the absence of gross changes in the eye disease, the general health of the rabbits rapidly deteriorated following initiation of therapy with corticotropin. They lost weight, developed diarrhea, became listless and apathetic, and one became partially paralyzed in his hindquarters. Three died within thirty days of the start of therapy, and the other 2 were destroyed because of the seriousness of their systemic illness. The change from good health to bad was a very impressive one in all of the 5 animals and it was not observed in the controls.

Follow-up observation in two rabbits whose infected eyes were treated early with corticotropin: Two animals whose fourteen-day treatment with corticotropin was started five days after ocular infection were observed for six months thereafter. At the end of that period the advanced ocular disease was identical grossly with that present in the 2 controls. The eye in each was replaced by a contracted mass of yellow-white material, well organized and without sign of inflammation. Furthermore, after the first month the health of the animals gradually returned to normal; they gained weight, became active, and looked well.

The ability of the animal to mobilize its defenses and to localize the ravaging disease appeared slowly after cessation of corticotropin. The rapidly progressive disease manifested by all animals during seven to fourteen days of hormone administration continued unabated for approximately two weeks thereafter. After twenty days, the inflammatory process was less acute and beginning organization was noted. Bulging from glaucoma and empyema gradually subsided, pus became more viscid, and vascular injection less intense. After sixty days the localization was well defined, and fibrosis and contraction of the residual

optic tissue had begun. The hormonal effect apparently persisted for at least two weeks after its cessation, and the subsequent course of the advanced disease simulated that in the untreated control. In figure 3 may be seen the appearance of the eye in figure 2 six months later. The contracted orbit, similar to the process in the controls, may be seen.

Cortisone-treated rabbits: Seven animals received cortisone for fourteen days. Two received the hormone prophylactically for twenty-four hours, 2 received first dosage on the day of ocular infection, and 3 received the hormone six months after establishment of advanced optic disease. The dosage of cortisone in 4 animals was 2.5 mg. daily and, in the 3 treated after six months, a total daily dose of 10 mg. was administered.

The disease in only one of the animals treated early with cortisone paralleled the infection in the corticotropin group. In this animal, ravaging ocular tuber-

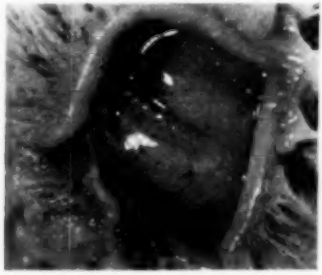

FIG. 3. Photograph of eye taken six months after termination of therapy with corticotropin. This is the eye shown in figure 2 and the photograph is dated September 18, 1951. Marked fibrosis and contraction are noted and the anterior chamber is replaced by a mass of thick, yellow, caseous material well localized within the chamber.

culosis was apparent within one week, and after two weeks the anterior eye was destroyed. Infection in the other 3 was not the same.

Figures 4*A*, *B*, *C*, and *D* show the course of ocular disease in one of these 3 latter animals and is typical of the disease in all. The rabbit received cortisone within twenty-four hours of the first inoculation of acid-fast bacilli. On the sixth day (figure 4*A*) ocular tuberculosis had progressed to the moderately advanced stage with acute iritis, cloudy chamber fluid, and a small, centrally placed, well-defined tubercle. At the end of the treatment period, the accessory evidences of acute inflammation had subsided but the tubercle in each was larger and more yellow (figure 4*B*). Subsequently, in the next four weeks, the eye became totally destroyed. Figure 4*C* shows the beginning of this change seven days after cessation of cortisone. There was no appreciable difference in the disease of the animals which received prophylactic cortisone from that which developed in animals which received their first hormone on the day of infection. Neither was there significant difference between the treated and the control eyes. Figure 4*D* shows the eye three months later, and the infection at that time was essentially similar to that in the controls.

Cortisone in the larger dosage did not, in any way, alter the fibrotic disease in the 3 animals whose infection was of six months' duration.

Immunological response in rabbits infected intra-ocularly with H37Rv and treated with adrenal hormones: In all of the 39 rabbits in the series, the cutaneous reaction to tuberculin was tested with 25 per cent or 50 per cent of OT before, during,

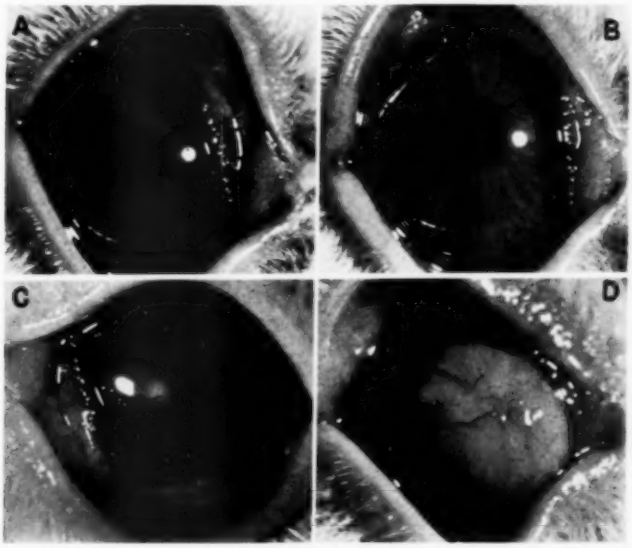

FIG. 4 *A*. Photograph of rabbit eye (A-26) dated May 21, 1951, taken five days after start of cortisone therapy and five days after ocular infection. Mild iritis, edema of cornea, and a small off-center tubercle are seen.

B. Photograph of same eye taken seven days later (May 28, 1951) at the end of therapy with cortisone. Lesion has not extended appreciably, but edema and iritis are mildly worse.

C. Photograph of same eye taken nine days after "*B*" (June 6, 1951). Extension of the infection is obvious and the lesion is now at the moderately advanced stage. Ocular lesions in controls closely parallel these changes.

D. Photograph of same eye taken three months later (September 11, 1951). Anterior chamber is replaced by yellow mass of hard, caseous material which is well localized. Organization of lesion is evident, new vessels from the limbus are seen.

and shortly after exposure to adrenal hormones. Eighteen animals had positive reactions prior to the first administration of hormones. These remained positive throughout the treatment period and for six months thereafter in those animals which were permitted to live for that period. The remaining rabbits had negative tuberculin reactions at the time ocular disease was first established. In these animals, the cutaneous reactions became positive between thirty and forty days after infection, an interval identical with that observed previously in animals which received no hormone (1). Neither adrenal hormone influenced

the development of cutaneous hypersensitivity to OT or changed the already positive reactions.

The administration of the adrenal hormones did not influence the hemagglutinin reaction in the infected animals. In rabbits whose optic disease had been established six to eight months prior to exposure to the drugs, levels of circulating agglutinins had become stabilized at titers above 1:32, and therapy caused no change in the levels. Serial determinations twice weekly during administration of either hormone, and for six months thereafter, revealed titers that did not vary more than one tube dilution. Measurable concentrations of hemagglutinins appeared in the infected rabbits treated early with both corticotropin and cortisone at the same time as they appeared in the control animals.

The adrenal glands of 16 rabbits, 8 treated and 8 controls, were weighed immediately after death and the content of vitamin C was measured. The average wet weight of one control gland was 190 mg., with a range of 91.1 to 438.8 mg. The average wet weight of a single treated gland was 273 mg., with a range of 100.1 to 478.5 mg. Only one of the sixteen glands in the treated group weighed less than 160 mg., while seven weighed under 160 mg. in the control group.

Ascorbic acid content of these same thirty-two glands was measured. In the control series, the average ascorbic acid content of the adrenal gland was 205 γ per gm. (wet weight), with a range of 152 to 248 γ of vitamin C. Eight glands of the sixteen were heavier than the average. The vitamin C content of the treated group averaged 259 γ per gm. of gland, and the range was 204 to 387 γ. Eight glands were lighter than the average.

Post-mortem findings in animals which received corticotropin or cortisone: Autopsies were performed upon 14 rabbits: 3 control animals, 9 treated with corticotropin, and 2 treated with cortisone. Serial sections of one-half of each infected eye and seven or eight isolated sections of lung and spleen were stained both by hematoxylin-eosin-azure and hematoxylin-carbol-fuchsin methods.

Upon gross examination at autopsy, multiple small tuberculous lesions in the lungs were noted in 6 of the 9 animals treated early with corticotropin and in one of the 3 control animals. The spleen alone was involved in a seventh animal treated with corticotropin. The presence of active tuberculosis in these areas was proved by finding tubercle bacilli in large numbers on smear and by culture. Neither animal treated with cortisone had metastatic disease at autopsy. Tuberculosis was found in all eyes examined. The extensiveness of the lesions varied, but all were advanced, all were acutely inflamed, and the anterior chambers were uniformly filled with pus.

The findings upon microscopic examination of the sections of eyes were striking. Three differences were noted between the lesions in animals which had received hormones and in those of the controls. In the former group the acuteness of the inflammatory response was impressive, there was a notable absence of fibroblasts, and myriads of tubercle bacilli were seen in all sections. Figures 5.1 and *B* (same eye as pictured during life in figure 1) are two low-power photomicrographs, representative of the type of lesion which was characteristic of all sections studied. There was almost complete destruction of all normal architecture behind the intact but inflamed conjunctiva. The cornea

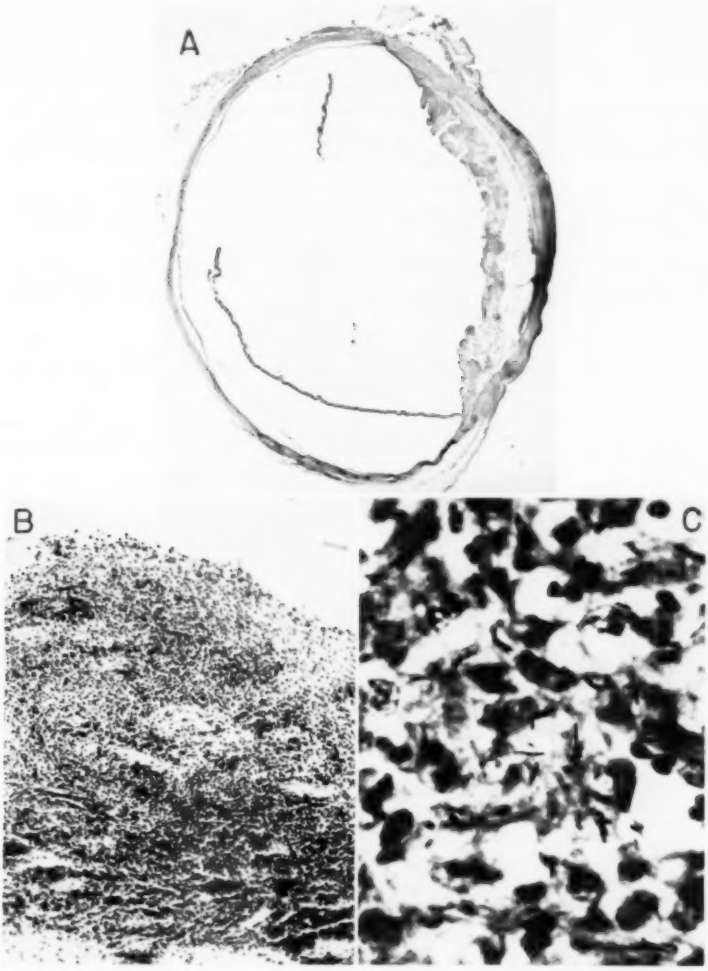

FIG. 5 *A*. Cross-section (× 12) of eye from corticotropin-treated animal whose ocular tuberculosis is of one month's duration. Eye is from animal pictured in figure 1. Posterior eye is normal; all tuberculous disease is anterior to ciliary body; all segments of the anterior eye are involved in the acute tuberculous process.

B. Photomicrograph (× 140) of acute tuberculous inflammation in region of ciliary body. The animal received corticotropin for fourteen days and the autopsy was performed two weeks after cessation of treatment. Majority of cells are polymorphonuclear. Small areas of tissue destruction are visible.

C. High-power photomicrograph (× 1650) of section shown in figure 5*B*. Tubercle bacilli are present in large numbers and their phagocytosis is evident.

was invaded, primarily by polymorphonuclear cells, was swollen to twice its normal size, and there was increased vascularity in it. Both the ciliary muscle and processes were completely replaced by acute inflammatory cells, and only a remnant of the iris remained. The majority of the cells in these areas were lymphocytes but at least 30 per cent were polymorphonuclear. Multiple small zones of tissue destruction were also evident in all segments of the ciliary body. No giant cells were observed, and there were only rare areas that conceivably

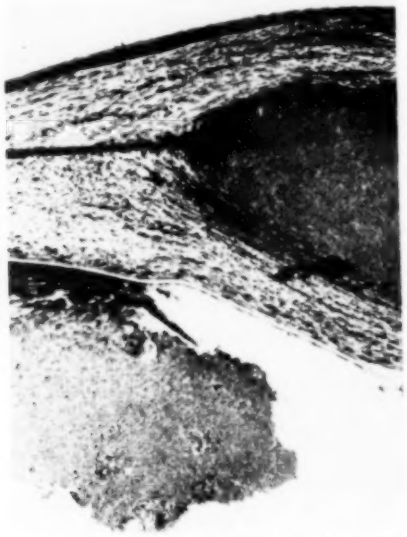

FIG. 6. Photomicrograph (× 78) of section of cornea and conjunctiva near ciliary body. Solitary tubercle in cornea is well localized. Material occupying anterior eye chamber is caseous but it also is well localized by band of fibrous tissue. Conjunctiva is normal. Section is taken from control animal whose tuberculosis was of one hundred days' duration prior to death.

could be called tuberculous granulomas. Fibroblastic activity was not observed anywhere in the eye.

The posterior portion of the eye was intact, though the vitreous was cloudy and viscid.

Figure 5*C* is a high-power enlargement of a photomicrograph of one of the acutely inflamed areas, stained with hematoxylin-carbol-fuchsin. Acid-fast bacilli are present in large numbers.

Figure 6 is a low-power photomicrograph of a control animal whose disease was of four months' duration at the time of death. The large caseous area in the anterior chamber has been well localized by a thin band of fibrous tissue. Simi-

larly, the isolated tubercle within the cornea itself is well localized and the surrounding cornea is little involved.

DISCUSSION

From the above observations there is direct evidence that corticotropin alters the course of early tuberculosis in the rabbit's eye. This effect varies and, in the main, is dependent upon the timing of the administration of the hormone in relation to the institution of the ocular disease. When given prior to infection, tuberculosis is suppressed and gross ocular lesions do not develop until the animal's adrenals are no longer stimulated. During the period of artificially induced stress, organisms deposited in the eye remain viable and are presumably reproducing, although this point was not definitely studied. When ocular disease is established at the time of, or within fourteen days of, the first dose of corticotropin, the course of the tuberculosis is speeded, more rapid destruction of the eye occurs, and metastatic tuberculosis very often develops.

Despite these initial and impressive deleterious effects during the administration of corticotropin, the subsequent course of ocular tuberculosis due to human-strain tubercle bacilli is not significantly different from that in untreated controls and follows the course expected in a tuberculin-sensitized animal (1). Although peripheral dissemination occurs and although the eye becomes destroyed during exposure to corticotropin, the animal survives, gains weight, and never becomes morbid if the hormone is stopped after two weeks. Three months after withdrawal, the eye disease parallels that in the control. It seems plausible to presume that, whatever the effect of adrenal stimulation upon tuberculosis, that effect ceases promptly when the stress is reduced. No explanation for the immediate inhibition of the localizing defenses, which are generally present in the rabbit to a great degree in human-strain tuberculous infections, can be offered; but similar observations have been made upon other animals, including man, with other bacterial infections (7).

Once the tuberculous infection has become stabilized in the rabbit eye, with fibrosis and complete gross localization, neither corticotropin nor cortisone has the ability to alter it in that area. It can be presumed, therefore, that neither hormone can affect fibrotic tissue or tear down local disease which is of quiescent nature (6, 8). Nevertheless, the animal as a whole suffers from the administration of corticotropin when there is local, apparently arrested tuberculosis. Distant acute disease develops and the animal succumbs to a septic infection despite the inapparent change in the mother lesion.

In the studies presented, cortisone had less effect upon the course of tuberculosis in the rabbit eye. It is very possible that the dosage of cortisone used was insufficient. Because of this, no conclusions can be made that cortisone and corticotropin act in different fashion upon tuberculosis in the rabbit. The doubt remains that cortisone is as capable of inhibiting the formation of new inflammatory tissue as that which occurs with whole gland stimulation by corticotropin. Indeed, it is logical to presume that in these sets of circumstances corticotropin is the more powerful deterrent to early adequate healing.

Maximal stimulating effect upon adrenal glands was obtained from the dosage of corticotropin utilized. Hypertrophy of the adrenal was universal and the ascorbic acid level was statistically greater in these than in all controls. This provides evidence that in the present studies the glands were being stimulated to a marked degree and that the observations recorded were caused by that stimulation.

Excessive adrenal stimulation did not change the two immunologic characteristics of tuberculosis in the rabbit that were measured, i.e., the skin reaction to Old Tuberculin and circulating hemagglutinins. Whatever the action of corticotropin upon the course of ocular tuberculosis in the rabbit may be, that action certainly does not involve those two defenses of the animal. This is not an unusual phenomenon, for similar observations have been reported in other bacterial infections in other animals as well as in man (7, 8).

Precisely how adrenal stimulation—or perhaps corticotropin per se—acts upon local infection with tubercle bacilli remains totally speculative. The course of early ocular tuberculosis in the rabbit is affected by corticotropin, however, and the alteration is a deleterious one. The normal and early response of the rabbit eye infection with human-strain tubercle bacilli is generally an effective response. It is not so when under the influence of the adrenocorticotropic hormone.

SUMMARY

Corticotropin (ACTH) in dosage which assures maximal response upon the adrenal glands of rabbits alters the course of an ocular infection with human-type tubercle bacilli. For the most part, the alteration upon early ocular tuberculous infections is a deleterious one. Upon established disease, the local effect is less demonstrable, but deleterious effects upon animals as a whole were noted. The change which corticotropin exerts upon early tuberculous disease in the rabbit eye is characterized by the animal's reduced ability to respond with standard inflammation and to localize the infection.

Cortisone, in the dosage used, did not alter to the same degree the course of ocular tuberculosis in the rabbit.

SUMARIO

Empleo del Ojo del Conejo como Tejido para el Estudio de la Tuberculosis. III. Efectos de las Hormonas Suprarrenales sobre la Evolución de la Enfermedad y sobre Ciertas Respuestas Inmunológicas

La corticotropina (HACT), a dosis que asegure la obtención de la respuesta máxima en las glándulas adrenales de los conejos infectados intraocularmente con bacilos tuberculosos humanos, altera la evolución de la infección. En su mayor parte, la alteración observada en las infecciones tuberculosas incipientes es perjudicial. En la enfermedad establecida, el efecto local es menos demonstrable, pero para el animal en conjunto, el efecto sigue siendo malo. La modificación que la corticotropina introduce en la enfermedad incipiente se caracteriza por la menor capacidad del animal para reaccionar con la inflamación habitual y para localizar la infección.

La cortisona, a las dosis usadas y que pueden haber sido insuficientes, no alteró con tanta intensidad la evolución de la tuberculosis ocular en el conejo.

RESUME

L'utilisation de l'oeil du lapin pour l'étude de la tuberculose: III. Action des hormones surrénales sur le cours de l'affection et sur certaines réactions immunologiques

L'hormone corticotrope (ACTH), aux doses qui assurent une réaction maximale de la glande surrénale du lapin infecté, par voie oculaire, avec des bacilles tuberculeux de souche humaine, modifie le cours de l'infection. Dans l'infection tuberculeuse au début, cette action est en grande partie nocive. Sur la maladie établie, l'effet local est moins démontrable, mais pour l'organisme animal, dans son ensemble, l'effet est encore mauvais. La modification apportée par l'hormone corticotrope, dans la phase initiale de la maladie, se caractérise par une diminution de la capacité de l'animal à réagir sous la forme de processus inflammatoires standards et à localiser l'infection.

La cortisone, aux doses utilisées, et qui pourraient avoir été insuffisantes, n'a pas altéré au même degré le cours de la tuberculose oculaire chez le lapin.

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TRIANGULAR HILAR SHADOWS[1]

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INTRODUCTION

The significance of shadows in chest roentgenograms of infants and young children is frequently difficult to evaluate. This is due, in part, to the rapid respiratory excursions and difficulty in posturing and maintaining the posture of the infant or child while the roentgenogram is being made. Attention has been called to some of the pitfalls and difficulties by Shapiro and Bell (1), Farrell (2), and Caffey (3).

One of the perplexing problems in the interpretation of shadows observed in roentgenograms of the lungs has been the significance of widened mediastinums and triangular shadows at the hila of the lungs. The nature and significance of shadows of this type are equivocal. The work of Harvey (4) and Harvey and Bromer (5) has clarified the problem to some extent. It has become evident, however, that a number of disease entities can produce the lesion in question. In general, the conclusion has been reached that tuberculosis is a relatively infrequent cause.

Few articles dealing with this problem have been published by physicians who specialize in diseases of the chest. The problem has been recognized and dealt with more frequently by the general and pediatric radiologist. With increase in survey methods, it is expected that the phenomenon will be noted more frequently. Continued accumulation of data is needed to remove indecision in the disposition and care of the abnormality.

PLAN OF INVESTIGATION

A unique opportunity to find, observe, and follow individuals showing such lesions arose in the course of study of roentgenograms of a group of 3,008 persons, ranging in age from birth to twenty years of age. This group of persons was included in an investigation initiated in 1936 by the Office of Indian Affairs and The Henry Phipps Institute, University of Pennsylvania (6), on the value of BCG vaccine in control of tuberculosis. Of this group of 3,008 persons, 1,551 received an intracutaneous injection of BCG vaccine, while 1,457 received an intracutaneous injection of physiological saline solution and served as controls. The subjects were examined roentgenographically and tested for cutaneous hypersensitivity to tuberculoprotein each year for an eleven-year period. Chest roentgenograms of these cases have been carefully reviewed by the writer to determine the incidence of pulmonary lesions. Interpretation of the individual films was made without knowledge whether the person concerned had received BCG or was a cutaneous reactor to tuberculoprotein.

[1] From Branch of Health, Office of Indian Affairs, U. S. Department of the Interior, Washington, D. C., and The Henry Phipps Institute, University of Pennsylvania, Philadelphia, Pa.

OBSERVATIONS

In the course of the study, 10 cases were found in which the films showed an abnormal triangular hilar shadow. A typical case is illustrated in figure 1. A correlation of a number of factors noted in the persons concerned is presented in table 1. In 3 cases there was sufficient evidence to warrant a diagnosis of tuberculosis. Two persons were in the control group and one was in the vaccinated group. A pre-existing lesion was noted in one case of tuberculosis in the control group and in the case of tuberculosis in the vaccinated group.

FIG. 1. N. W. Six years old. Roentgenogram of chest reveals right superior mediastinal widening with horizontal border projecting into horizontal fissure.

Study of distribution of cases by sex revealed only one case in a male. This may well be chance or coincidence, since comparison with Harvey's (4) 9 cases showed 6 cases in males and 3 in females.

Investigation with respect to age revealed no cases after the age of nine years. The earliest case was that of a child of three months. An effort was made to determine the significance of history of contact in these patients. Four of the 10 patients revealed a definite history of contact; in 2 of them, tuberculosis developed. Although the figures are far too small for definitive evaluation, the occurrence of 2 cases in 4 known contacts appears significant.

TABLE 1

FACTORS NOTED IN CASES OF TRIANGULAR HILAR DENSITIES

NAME	AGE IN YEARS	TRIBE	BCG (B) OR CONTROL (C)	HISTORY OF CONTACT	YEAR OF LESION	SITE	ANNUAL TUBERCULIN TEST	NUMBER OF MONTHS DURATION*	DIAGNOSIS	TUBERCULIN REACTION IN MM.
S. G.	8	Arapaho	C	+	40† 41	Left	39 40 41 42 0 2 3 3 5 2 2 2	36 18	Tuberculosis	18 x 20 x 2
F. B.	6	Shoshone	C	−	41	Right	39 40 41 42 0 0 ± 1 5 5 5 5	25 14	Thymus?	Erythema without induration 8 x 9
M. W.	8	Shoshone	C	−	42	Right	(37–41) 42 43 0 3 2 5 5 2	54 11	Tuberculosis	47 x 46 x 3
T. M.	3	Alaska	C	−	40	Right	(37–42) 43 47 0 3 2 5 2 2	13	Thymus? (Calc. 1947)	0
B. B.	1	Alaska	C	+	38	Right	38 39 40 (42–44) 47 0 1 0 N.T. 0 5 5 5 5	11	Thymus?	0
G. G.	9	Shoshone	B	−	38	Left	36 37 38 39 (40–43) 0 2 2 1 2 5 5 2 2 2	24	Thymus?	12 x 10 x 1
N. W.	6	Alaska	B	+	37† 42	Right	37 38 40 41 42 43 47 0 2 3 1 1 2 2 5 5 5 2 2 2 2	111 11	Tuberculosis	20 x 23 x 2

V. W	2	Alaska	B	−	39	Right	37 $\frac{0}{5}$ 38 N.T. 39 $\frac{2}{5}$ 40 $\frac{2}{5}$ 43 $\frac{1}{2}$ (44–47) $\frac{1}{2}$	12	Thymus?	7 x 5 x 1
L. D.	$\frac{5}{12}$	T. Mt.	B	+	39	Right	39 $\frac{1}{5}$ (40–43) $\frac{2}{5}$ 44 $\frac{2}{2}$	12	Thymus?	6 x 7 x 1
R. D.	$\frac{3}{12}$	T. Mt.	B	−	39	Right	39 $\frac{1}{5}$ (40–44) $\frac{2}{5}$ 46 $\frac{2}{5}$	12	Thymus?	5 x 5 x 1

* Numerator is duration of lesion; denominator, duration of triangular shadow. Numerator represents severity of reaction (0, 1+, 3+). Denominator represents dosage of tuberculin (5 = 0.005 mg.; 2 = 0.00002 mg.)

† Cases "S. G." and "N. W." had pre-existing lesions.

The lesion was usually on the right side, as noted by Harvey (4). In his series, 7 were on the right side and 2 on the left. The series here presented revealed 8 patients with characteristic lesions on the right side and 2 on the left. Not one case of bilateral triangular density was noted, although this has been reported. There were 2 deaths in Harvey's (4) group, both apparently of nontuberculous etiology; no deaths occurred in the cases in the present series within the period of observation.

The annual tuberculin tests are recorded in table 1. A distinct correlation with the tuberculin reaction is noted in the 2 cases of the control group. Tuberculin test correlation in the vaccinated group is difficult because BCG vaccination itself elicits tuberculin sensitivity. It has been noted, however, that an increase in the tuberculin sensitivity occurs in vaccinated persons who develop pulmonary tuberculosis. An increase in sensitivity was noted in the case of "N. W.," whose lesion occurred in 1942 and in whom a sputum positive for tubercle bacilli was found in March, 1943.

It is the general opinion that the lesion is of relatively short duration. The longest period of observation of the triangular hilar shadow in the present series was two years. A longer duration of the lesion has been noted in the German than in the American literature. Difference in duration of the abnormality might be a clue in the differential diagnosis. Although the triangular density cleared, usually within one year, an accompanying hilar widening or parenchymal lesion persisted for longer periods. It is suspected, therefore, that persistence of an abnormality in association with the triangular density may justify a diagnosis of tuberculosis.

No correlation with the geographic distribution of the patients was evident.

In general, radiologists have believed that these hilar abnormalities are usually benign and probably nontuberculous. The occurrence of anterior mediastinal lymph node tuberculosis has been considered unusual. Cases with such findings have been presented by Buckley (7), Reyher (8), and Harvey and Bromer (5).

It is concluded from the present investigation that a diagnosis of tuberculosis should be entertained if there is a history of contact with tuberculosis, if skin sensitivity to tuberculin is noted, and if a coinciding hilar node or parenchymal lesion persists for more than two years.

SUMMARY

In a series of chest roentgenograms of 1,551 BCG-vaccinated persons and 1,457 controls, 10 cases of hilar triangular shadows were observed. In 3 of these cases, unmistakable pulmonary tuberculosis developed.

In the majority of cases in this series, as well as in series previously reported, the lesion was on the right side. No instances of bilateral involvement were seen.

In 2 of the 3 patients with tuberculosis, there was a history of contact with tuberculosis. In all 3 (2 control and 1 vaccinated), the tuberculin test was positive.

No relation was noted in geographic origin of the subjects of the study. Nine of the 10 cases discovered were in males. Since previous studies have revealed no such sex distribution, this finding is probably a coincidence.

In the presence of triangular hilar shadows, it is believed that a diagnosis of tuberculosis should be considered if there is a history of contact with tuberculosis, if skin sensitivity to tuberculin is noted, and if an existing hilar node or parenchymal lesion persists for two or more years.

SUMARIO

Sombras Triangulares del Hilio

En una serie de radiografías torácicas de 1,551 personas bcegetizadas y 1,457 testigos, observáronse 10 casos de imágenes hiliares triangulares. En 3 de estos casos, apareció tuberculosis pulmonar indudable.

En la mayoría de los casos de esta serie, así como en series previamente descritas, la lesión quedaba en el lado derecho. No se observaron casos bilaterales.

En 2 de los 3 casos de tuberculosis, había antecedentes de contacto con tuberculosis. En todos (2 testigos y un vacunado), la reacción a la tuberculina resultó positiva.

No se notó relación con la procedencia geográfica de los sujetos. Nueve de los 10 casos de sombras hiliares fueron en varones. Visto que ninguno de los estudios anteriores ha revelado tal predominio en un sexo, este hallazgo constituye probablemente una coincidencia.

Parece que debe considerarse el diagnóstico de tuberculosis si existe historia de contacto con la dolencia, si se descubre cutisensibilidad a la tuberculina y si una lesión presente en el parénquima o en los ganglios hiliares persiste dos años o más.

RESUME

Ombres hilaires triangulaires

Des ombres hilaires triangulaires ont été observées 10 fois dans une série de clichés radiographiques comprenant 1.551 sujets vaccinés par le BCG et 1.457 sujets-témoins. Dans trois de ces cas, le développement de la tuberculose pulmonaire fut nettement établi.

Dans la majorité des cas de cette série, comme dans les séries précédemment rapportées, la lésion était située à droite. Il n'a pas été observé de lésions bilatérales.

Dans 2 de ces trois cas de tuberculose, il y avait eu exposition à la contagion. Dans les trois cas (2 sujets témoins et un sujet vacciné), le "tuberculin-test" était positif.

Aucune relation n'a été observée quant à l'origine géographique des sujets étudiés. Sur les 10 cas, neuf étaient de sexe masculin. Etant donné que les études antérieures n'ont pas révélé cette répartition selon le sexe, ce résultat est considéré comme une coincidence.

L'auteur pense que le diagnostic de tuberculose doit être envisagé s'il existe une histoire d'exposition à la tuberculose, si la cuti-réaction à la tuberculine est positive et en présence de ganglions hilaires ou de lésions du parenchyme persistant pendant deux ans ou plus.

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STREPTOMYCIN IN GUINEA PIGS WITH DISCRETE CHRONIC TUBERCULOUS LESIONS[1,2]

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INTRODUCTION

Early in the studies in this laboratory on the streptomycin treatment of acute tuberculosis in guinea pigs, it was noted that a subcutaneous abscess at the site of inoculation, which did not ulcerate and discharge its contents, would remain intact during many months of continuous treatment. This observation, as well as reports by other investigators (1, 2) on the finding of residual areas of necrotic tissue in lungs of patients after prolonged antimicrobial therapy, stimulated one of the present writers to question whether present-day laboratory methods are adequate for the evaluation of antituberculous agents prior to their use in humans (3).

It has been definitely established that early acute tuberculous disease in both animals and humans responds favorably to streptomycin or the combination of streptomycin and para-aminosalicylic acid (PAS). The present report is concerned with one phase of a long-range project designed to study the effect of antimicrobial therapy on experimentally produced necrotic lesions which resemble those of chronic pulmonary phthisis in humans. The plan was also to study the incidence of drug-resistant strains of *M. tuberculosis* which appeared during long-term streptomycin treatment of such chronic necrotic lesions, in view of the failure to demonstrate such strains in any but a very small percentage of guinea pigs with acute tuberculous lesions even after prolonged streptomycin administration (4).

MATERIALS AND METHODS

Chronic discrete tuberculous lesions to be treated with streptomycin were produced in guinea pigs by the following two methods:

Animals allowed to relapse after a preliminary short course of streptomycin: A group of guinea pigs, weighing between 400 and 700 gm., was infected subcutaneously in the inguinal region with 0.05 mg. (dry weight) of *M. tuberculosis* H37Rv. Treatment with streptomycin was begun 41 days later. Three different dosages were used, namely, 7.5, 15, and 30 mg. per day intramuscularly. Since there was no significant difference in behavior of the animals treated with one dose as compared with the others, they will be considered as a single group. Treatment was continued for only 90 days, after which the animals were allowed to go untreated for 165 days. At the end of this time, when they were presumed to

[1] From the Trudeau Laboratory of the Trudeau Foundation for the Clinical and Experimental Study of Pulmonary Disease, Trudeau, New York.

[2] This study was aided by grants from the Division of Research Grants and Fellowships of the National Institutes of Health, Public Health Service.

have developed the desired chronic lesions, the animals were divided into two groups: one group of 24 remained untreated and was followed until death; the other group of 11 was re-treated with streptomycin, 15 mg. once daily, intramuscularly, for 300 days.

Animals with increased resistance to infection produced by vaccination: Previous experience has indicated that a slowly progressive nodular type of tuberculosis may be observed in guinea pigs whose resistance to infection has been increased by vaccination with attenuated living tubercle bacilli. Two groups of guinea pigs were vaccinated with the attenuated human strain, H37Ra (5), by injecting 2.5 mg. subcutaneously three times at three-day intervals:

Group 1 (35 animals), vaccinated one year previous to infection
Group 2 (20 animals), vaccinated one month previous to infection

The two groups of vaccinated guinea pigs, together with 10 unvaccinated controls, were infected subcutaneously with 0.08 mg. (dry weight) of the virulent *M. tuberculosis* H37Rv strain. Ninety-six days after infection a representative sample of the vaccinated pigs was sacrificed for gross and microscopic study; the following day the remaining vaccinated animals were started on streptomycin treatment, 15 mg. once daily intramuscularly. Treatment was continued for approximately 300 days, after which all surviving guinea pigs were sacrificed.

RESULTS

Animals with Post-Treatment Relapsing Tuberculosis

One hundred and sixty-five days after a preliminary ninety-day course of streptomycin treatment, the type of tuberculous disease present in this group of guinea pigs was revealed by 6 animals which died of nontuberculous infection within a few days of the time re-treatment was started. Four of the 6 revealed chronic-type lesions characterized by large discrete areas of caseation and cavity formation in the lungs and by large nodular tubercles in the liver and spleen. Two showed scattered small tubercles in the lungs; large, irregular, hard, yellow areas in the liver; and very large purple spleens containing irregular necrotic areas. All of the animals had extensive progressive tuberculosis.

Of the 24 guinea pigs that remained untreated, 11 died of streptococcal pneumonia or other intercurrent nontuberculous infection, and 13 died of progressive tuberculosis 87 to 336 days after the original ninety-day course of streptomycin. All 13 in the latter group showed chronic-type lesions, with large areas of caseation and cavitation in the lungs and large isolated nodular tubercles in the spleen.

Of the 11 re-treated animals, most of which presumably harbored chronic-type tuberculous lesions, 2 died within five days of the onset of the second course of streptomycin. No cause for these deaths could be demonstrated. Six animals died of intercurrent infection; 3 remained alive and were sacrificed after 300 days of re-treatment. In table 1 are presented the bacteriologic and pathologic (gross and microscopic) findings of the re-treated animals, excluding the 2 which died prematurely.

Five of the 6 guinea pigs which died of intercurrent infection showed very few residual tuberculous lesions, and their deaths were obviously caused by the nontuberculous disease. The sixth animal (No. 370), however, demonstrated active and progressive lesions in the lungs and spleen. This animal had paralysis of the hindquarters and steady loss of weight for several weeks before death. The brain showed no abnormal findings, either macroscopically or microscopic-

TABLE 1

Findings in Guinea Pigs Re-treated with Streptomycin 165 Days After the Conclusion of a Preliminary Ninety-Day Course of Streptomycin

Guinea pig	Days of life†	Findings at autopsy*					Remarks	Streptomycin susceptibility of recovered tubercle bacilli‡
		Lungs	Liver	Spleen	Lymph Nodes	Total		
541	139	0	0	0	1+ Left inguinal enlarged and firm	1§	Died of pneumonia; abscess at site of infection	
365	139	0	0	0	1+ Left inguinal enlarged and firm	1	Died of pneumonia	
836	226	0	0	0 *Scars and peri-Malpighian body fibrosis*	0	0	Died of intercurrent infection	
370	235	3+ Many small tubercles. *Epithelioid tubercles without capsules and peripheral epithelioid cell pneumonia; some central caseation*	0 *Epithelioid tubercles in portal spaces*	3+ Normal size; many discrete tubercles. *Epithelioid tubercles with central necrosis; no scarring*	2½+ Hepatic and paratracheal very large and hard. *Epithelioid tubercles, caseation and scarring*	8.5	Hindquarters paralyzed; serous peritoneal and pleural fluid	1.0–2.5
402	250	0	0 *One epithelioid tubercle with central caseation*	1+ Normal size; one nodular tubercle seen. *One area active epithelioid tubercles; two foci residual caseous material*	2+ Left inguinal and iliac enlarged and hard. *Epithelioid tubercles and much scarring*	3	Milky pleural and peritoneal fluid, probably result of secondary infection	Culture negative
467	281	0	0 *Three minute epithelioid tubercles*	0 *Peri-Malpighian body fibrosis*	0 *One minute epithelioid tubercle*	0	Died of secondary infection	Culture negative
687	300	± Linear scars	0	1+ Several small nodular tubercles. *Marked peri-Malpighian body fibrosis*	1+ Left iliac caseous	2.5	Killed	1.0–2.5 (guinea pig subinoculation)

TABLE 1—*Continued*

GUINEA PIG	DAYS OF LIFE†	FINDINGS AT AUTOPSY*					REMARKS	STREPTOMYCIN SUSCEPTIBILITY OF RECOVERED TUBERCLE BACILLI‡§
		Lungs	Liver	Spleen	Lymph Nodes	Total		
295	300	± Linear scars. *One small scar*	± Tiny gray foci. *No evidence of tuberculosis*	0	0	1	Killed	Culture and guinea pig subinoculation negative
459	300	± Linear scars. *Two areas diffuse scarring*	0 *Slight portal lymphocytosis*	0 *Marked peri-Malpighian body fibrosis*	± Hepatic slightly enlarged	1	Killed	

* Microscopic observations indicated by italics.

† From the start of re-treatment with streptomycin.

‡ Blank space indicates no culture or guinea pig subinoculation attempted.

§ Amount of gross tuberculous disease, based on evaluation of 0 to 4+ for lungs, liver, spleen, and lymph nodes.

‖ Resistant to 1.0 γ, sensitive to 2.5 γ, of streptomycin per ml. of liquid Tween-albumin medium.

ally. The amount of tuberculosis was not believed to have been sufficient to cause death, which was presumed to be the result of an intercurrent nontuberculous infection. Tubercle bacilli cultured from the lungs and spleen were resistant to 1 γ, and susceptible to 2.5 γ, of streptomycin per ml. of liquid Tween[3] albumin medium. Direct culture of the organs on solid egg-yolk–potato–glycerin medium (American Trudeau Society medium) showed growth in the control tubes only and no growth in the tubes containing streptomycin in concentrations of approximately 3.5, 15, and 200 γ per ml.

The 3 guinea pigs killed after 300 days of re-treatment showed very little tuberculous disease. Two of the animals demonstrated nothing but tiny scars in some of the organs. One animal had several small nodular tubercles in the spleen and a small amount of caseation in the left iliac lymph node. Culture of the macerated organs of this animal was negative, but one of 2 guinea pigs inoculated with the same material developed progressive tuberculosis. The tubercle bacilli recovered from this subinoculated guinea pig proved to be resistant to 1 γ, and susceptible to 2.5 γ, of streptomycin per ml. of Tween-albumin liquid medium.

The scarring in the organs of the guinea pigs re-treated with streptomycin for 300 days may be seen in figure 1. These scars have none of the characteristics of tuberculous lesions, such as epithelioid cells, multinucleated giant cells, or caseation. They were presumed to be tuberculous in origin, however, since during the same year examination of the organs of some 800 guinea pigs from

[3] Proprietary name of the polyoxyethylene derivative of Sorbitan monooleate, obtained from the Atlas Powder Co., Wilmington, Delaware.

the same stock which had not been infected with tubercle bacilli failed to reveal similar lesions. This statement also applies to the fibrous scars around the Malpighian bodies seen in the spleens of the animals in the second experiment.

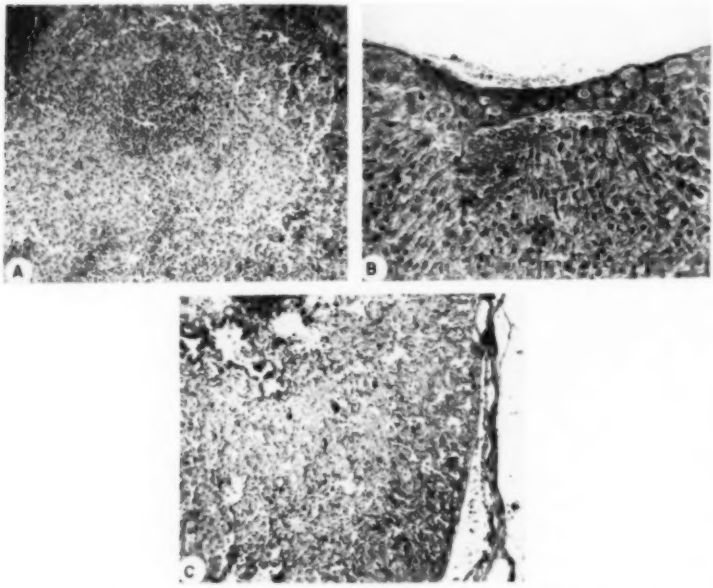

FIG. 1. Residual scars in guinea pigs after 300 days of re-treatment with streptomycin

A. No. 687 Spleen. Malpighian body, with wide peripheral zone of fibrous tissue. (100×)

B. No. 687 Liver. In the mid-portion there is a depression of the surface with collagenous thickening of the capsule. (200×)

C. No. 459 Lung. The alveolar structure is replaced by collagenous tissue, although the original pattern tends to be preserved. Pleural thickening is demonstrated at the right. (100×)

Vaccinated Animals

All of the 10 normal unvaccinated control guinea pigs were dead within 90 days after infection with the virulent organisms. On the ninety-sixth day, when the sample of vaccinated guinea pigs was sacrificed, the groups consisted of the following:

Group 1 (vaccinated one year previous to infection): Of the original 35 animals, 13 had died (6 with sufficient tuberculosis to explain death; 7 of intercurrent infection or unknown cause but with little tuberculous disease) and 22 remained alive.

Group 2 (vaccinated one month previous to infection): Of the 20 original members, 7 had died (only one of progressive tuberculosis) and 13 remained alive.

The 7 guinea pigs sacrificed from *Group 1* showed large, scattered, discrete, firm tubercles in the lungs, many with cavity formation. Microscopically, there were well-defined areas of caseation with thin peripheral zones of epithelioid cells and scattered lymphocytes. There was no fibrous tissue in the capsules. In many of the lesions the caseous material had excavated, leaving true cavities. In figure 2*B* may be seen two such cavities and the bronchial communication of one. On macroscopic examination, the livers showed a few large firm tubercles and, microscopically, scattered epithelioid tubercles in the portal spaces, some with central necrosis. Occasional areas of coalescing tubercles ranging up to 5 mm. in diameter were noted. There was also generalized bile duct proliferation, as is customarily seen in a tuberculous guinea pig liver. The spleens showed many large discrete nodular firm tubercles, most of which were cystic. Microscopically, large foci of caseation with liquefaction were observed, surrounded by thin zones of epithelioid cells. A few of these lesions had fibrous capsules, and numerous very large giant cells were present.

The 4 pigs sacrificed from *Group 2* (vaccinated one month previous to infection) showed the same type of lesions, but these were much less extensive. The lungs had few to many small, discrete, gray areas which, microscopically, were sharply defined epithelioid cell tubercles, with central caseation and no apparent capsules. The livers showed few to many small, round, gray areas and, microscopically, few scattered epithelioid tubercles in the portal spaces, with no visible necrosis. Grossly, the spleens demonstrated a few discrete, usually large, nodular tubercles. The organ was enlarged slightly or not at all. Microscopically, the spleens were largely replaced by small epithelioid tubercles, some of which had central caseation. In some areas the tubercles were becoming confluent, with a larger central focus of caseation. There was slight scarring, but the lesions were not encapsulated.

In figures 2 and 3 are presented representative microscopic sections of the organs of the vaccinated guinea pigs sacrificed 96 days after infection, illustrating the pathologic lesions described above.

On the ninety-seventh day after infection, the remaining guinea pigs in both groups (15 in *Group 1;* 9 in *Group 2*) were started on streptomycin in dosage of 15 mg. once daily intramuscularly. This regimen was continued for 300 days. In the group which had been vaccinated one year previous to infection, 2 animals died of nontuberculous pneumonia, 5 days and 32 days, respectively, after treatment was started. Both of these animals had advanced tuberculous lesions. Six animals died from causes other than tuberculous infection after 63 to 200 days of treatment. The organs of these animals showed either no tuberculosis or only a few small isolated lesions. Seven guinea pigs survived the full treatment period and were killed after 300 days.

Of the guinea pigs vaccinated one month previous to infection, one died of a central nervous system infection of unknown etiology after 32 days of treatment and still demonstrated advanced tuberculous lesions. One died of unknown cause after 176 days of treatment, with no evidence of tuberculosis in any of the organs. Seven animals survived and were killed after 300 days of treatment.

The pertinent findings in the 14 guinea pigs which survived to the end of the experiment are shown in table 2.

It may be seen from the table that the animals in *Group 2* (vaccinated one month previous to infection) showed no significant lesions in any of the organs. The only gross or microscopic evidences of infection were a slight fibrosis in the

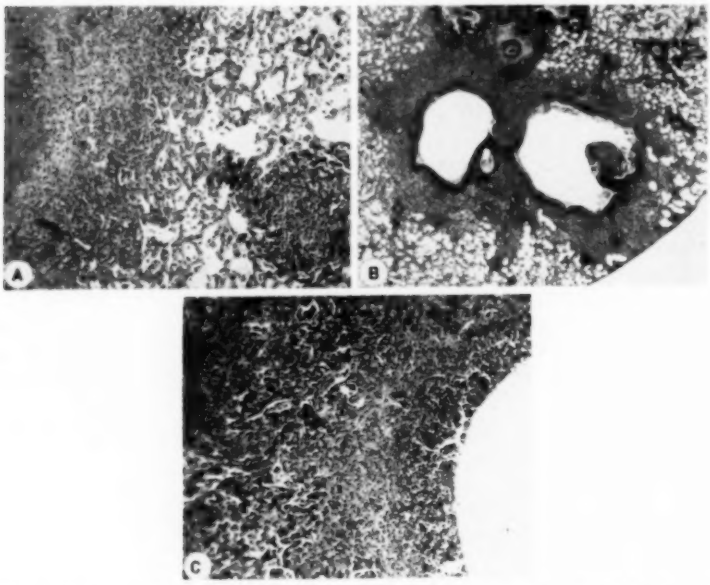

Fig. 2. Chronic lesions in vaccinated guinea pigs sacrificed 96 days after infection with virulent tubercle bacilli.

A. No. 56 Lung. Margin of a large caseous area (left) with epithelioid cells and lymphocytes in its wall. Collagen deposition is not apparent. At the right is a small epithelioid cell tubercle. (100×)

B. No. 98 Lung. Two pulmonary cavities are seen, together with the bronchus draining the cavity on the left (arrow). (32×)

C. No. 98 Lung. Higher power of the wall of the cavity on the left in "B." The cavity is lined by granular debris infiltrated with polymorphonuclear leukocytes. Beneath this lining is seen an epithelioid cell zone, with lymphocytic infiltration and slight scarring. (100×)

spleen of one animal and, in another, a small subcutaneous abscess at the site of infection. Culture of material from this local abscess yielded no growth. Among the animals which had been vaccinated one year previous to infection, however, 3 demonstrated large discrete lesions of an unusual type, whereas 4 animals revealed very little evidence of previous tuberculous infection. Gross and microscopic examination of the latter 4 animals revealed the following:

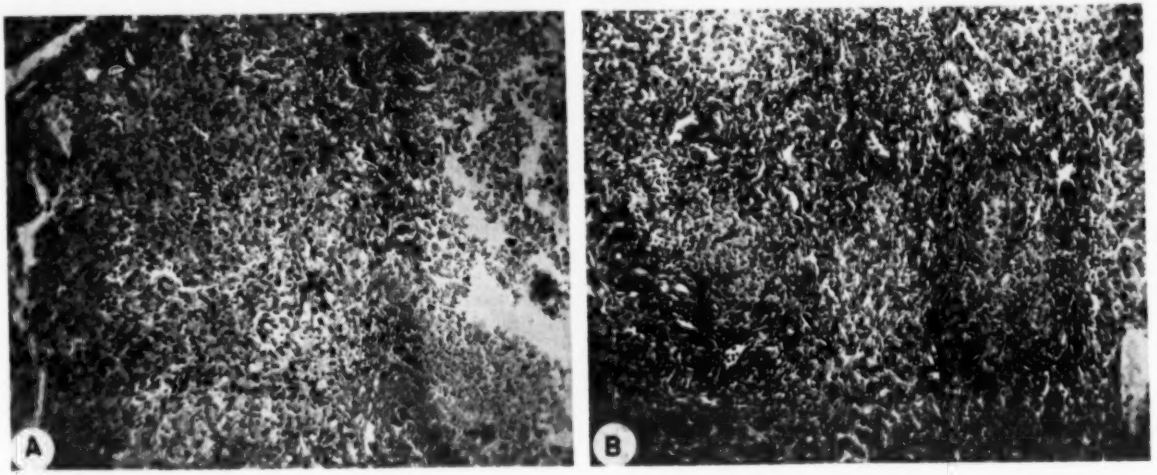

FIG. 3. Chronic lesions in vaccinated guinea pigs sacrificed 96 days after infection with virulent tubercle bacilli.

A. No. 56 Spleen. A "cystic" lesion in the spleen. The figure includes part of the wall of a large tuberculous lesion with partial liquefaction of the central caseous material (right). The wall is made up of epithelioid cells with lymphocytes and scattered multinucleated giant cells, with but little scar tissue. (100×)

B. No. 56 Liver. The picture reveals extensive involvement of the liver, with only thin zones of liver sinusoids remaining. The portal spaces are thickened and contain an excess of bile ducts and scattered unencapsulated epithelioid tubercles. (100×)

TABLE 2

Findings in Vaccinated Guinea Pigs Sacrificed After 300 Days of Streptomycin Treatment Which Started 97 Days After Infection

Guinea Pig	Vaccinated Before Infection	Findings at Autopsy*					Culture†	Susceptibility to Streptomycin
		Lungs	Liver	Spleen	Lymph Nodes	Total		
	months							
519	12	0	0	0 *Peri-Malpighian body fibrosis*	1+ *Many epithelioid tubercles*	1		
A99	12	± *Scarring, and encapsulated foci of caseation*	2+ Large abscesses	± *Peri-Malpighian body fibrosis*	0	3	Liver cyst #1: positive Liver cyst #2: positive Lung: negative	Susceptible Susceptible
A100	12	± *Scarring only*	0	0 *Peri-Malpighian body fibrosis*	1+ *Many epithelioid tubercles*	1.5		
A92	12	2+ Small gray tubercles; *epithelioid tubercles without capsules*	3+ Large abscesses	3+ *Peri-Malpighian body fibrosis and large spreading tubercles*	2+	10	Liver cyst: positive Spleen: positive Lung: negative	Susceptible Susceptible
A95	12	3+ Caseation and cavity formation	1+ Cystic nodules	0 *Peri-Malpighian body fibrosis*	0	4	Lung: negative Liver cyst: positive	Susceptible
A93	12	± *Scarring only*	0	0	±	1	Lung: negative	
A90	12	± *Scarring only*	1+ *Scarring; one epithelioid tubercle*	± *Peri-Malpighian body fibrosis*	0	2	Lung: negative	
A31	1	0	0	0 *Slight peri-Malpighian body fibrosis*	0	0		
A37	1	0	0	0	0	0		
A32	1	0	0	0	0	0	Local abscess at inoculation site: negative	
A33	1	0	0	0	0	0		
A76	1	0	0	0	0	0		
A72	1	±	0	0	0	0.5		
A74	1	0	0	0	0	0		

* Gross and microscopic findings. Figures indicate the amount of gross tuberculous disease, based on evaluation of 0 to 4+ for lungs, liver, spleen, and lymph nodes, with maximum total of 16 for each animal. Microscopic observations indicated by italics.

† Blank space indicates no culture attempted.

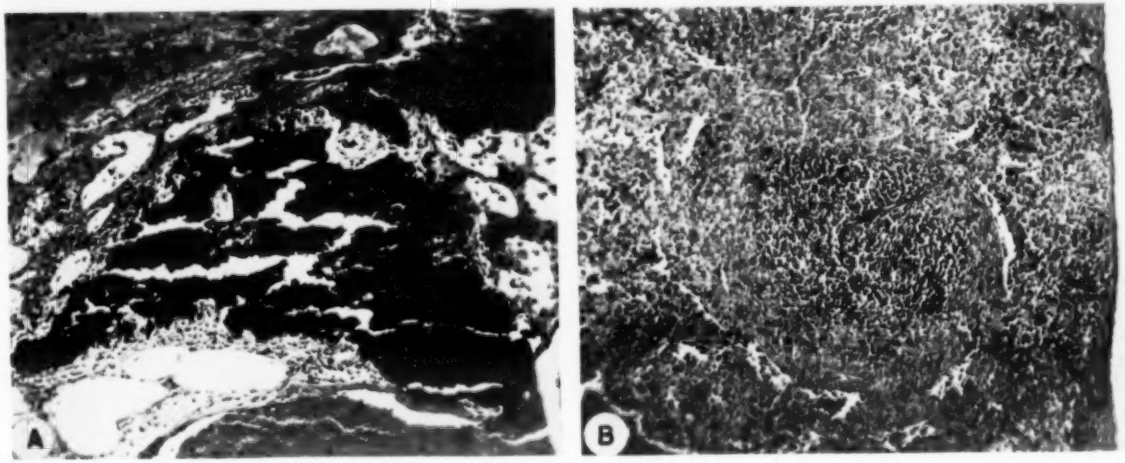

FIG. 4. Residual lesions after 300 days of streptomycin treatment in guinea pigs vaccinated one year prior to infection.

A. No. 93 Lung. The field is occupied by a large focus of calcified material, surrounded by a thin collagenous capsule with slight lymphocytic infiltration. Epithelioid and giant cells are absent. (100×)

B. No. 519 Spleen. Peri-Malpighian body fibrosis. Epithelioid and giant cells are absent. (100×)

White plaque-like scars and tiny gray puckered areas were evident grossly in the lungs. In one animal this organ revealed nothing abnormal microscopically and, in another, only a focus of scarring without evidence to indicate its origin. Two showed scars in which were small foci of caseation without epithelioid cells. A large focus of calcification (figure 4A) was seen in one. Gross examination of the livers revealed either nothing abnormal or merely a few firm gray areas. Microscopically, an occasional minute

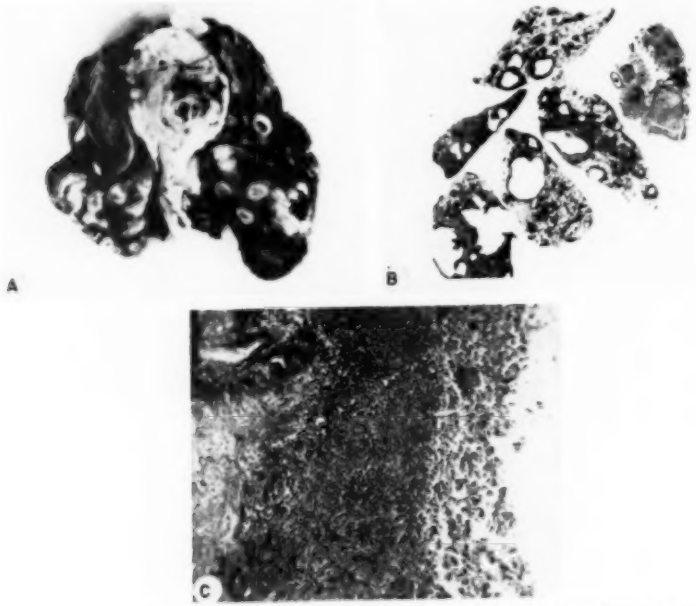

FIG. 5. Residual lesions after 300 days of streptomycin treatment in guinea pigs vaccinated one year prior to infection; from the 3 animals demonstrating unusual cystlike lesions.

No. A95. A. Lungs, showing thin-walled cysts.
B. Cut sections of lungs, showing large cavities.
C. Wall of pulmonary cavity. The cavity space is lined by macrophages and giant cells. The wall is made up of granulation tissue and collagenous tissue with well-defined epithelioid tubercles, one of which is seen in the center of the field. (100×)

scar in the portal area was seen. One animal showed an area of rather diffuse scarring which contained several slightly dilated bile ducts and one epithelioid tubercle. Grossly, the spleens appeared normal, but microscopically they all showed well-marked fibrous scars around the Malpighian bodies. As in the preceding experiment, the lesions were presumed to be residua of the tuberculous infection although there was nothing specific about the cellular reaction to indicate a tuberculous origin. The only evidences of tuberculosis in the lymph nodes were slightly enlarged firm inguinal nodes on the side of the

virulent infection. Microscopically, most of the inguinal nodes revealed confluent epithelioid tubercles with thin fibrous capsules and little or no caseation.

In figure 4 may be seen 2 residual lesions typical of those just described.

The other 3 animals presented the following picture:

In the lungs of one (No. A95), on the surface there were many large caseous abscesses and elevated bleblike structures containing clear fluid. Several cavities were seen in cut

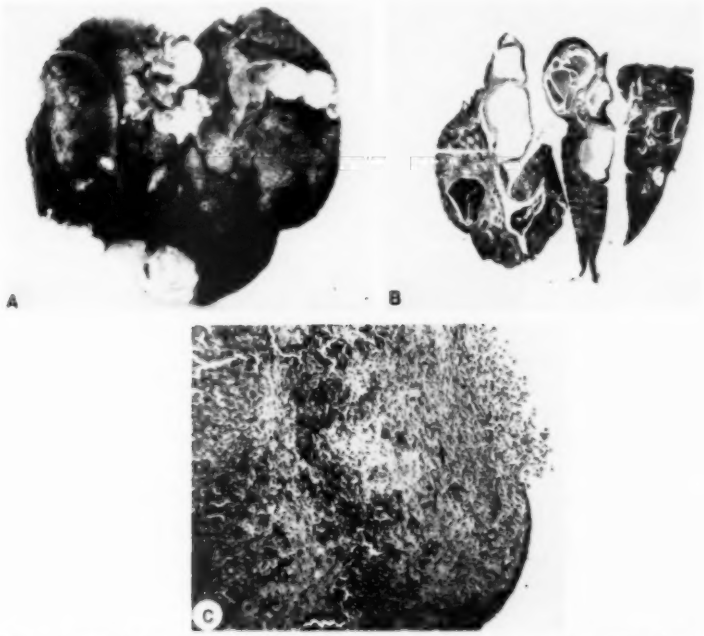

FIG. 6. Residual lesions after 300 days of streptomycin treatment in guinea pigs vaccinated one year prior to infection; from the 3 animals demonstrating unusual cystlike lesions.

No. A99. A. Liver showing thick-walled cystic abscesses.

B. Cut sections of same liver.

C. Wall of cystic abscess in liver. In the lower half of the field, the cyst is lined by epithelium resembling that of the bile ducts. Above this the epithelium is ulcerated and the surface is lined by a zone of macrophages. Beneath the lining is a zone of dense collagen. (100×)

sections. Microscopically, these were typical tuberculous cavities with caseous linings infiltrated with polymorphonuclear cells and macrophages, an epithelioid-cell zone, and a dense peripheral scar (figure 5). Another animal (No. A92) showed many small gray

discrete tubercles in the lungs, which, on microscopic examination, proved to be typical epithelioid tubercles, the majority without encapsulation.

The livers exhibited large, thick-walled, cystlike abscesses filled with creamy pus, which, on direct smear, contained very many acid-fast bacilli. The cystlike abscesses were lined with high cuboidal epithelium resembling that seen in the bile ducts. The walls were generally collagenous, but occasional strands of smooth muscle were also seen. In

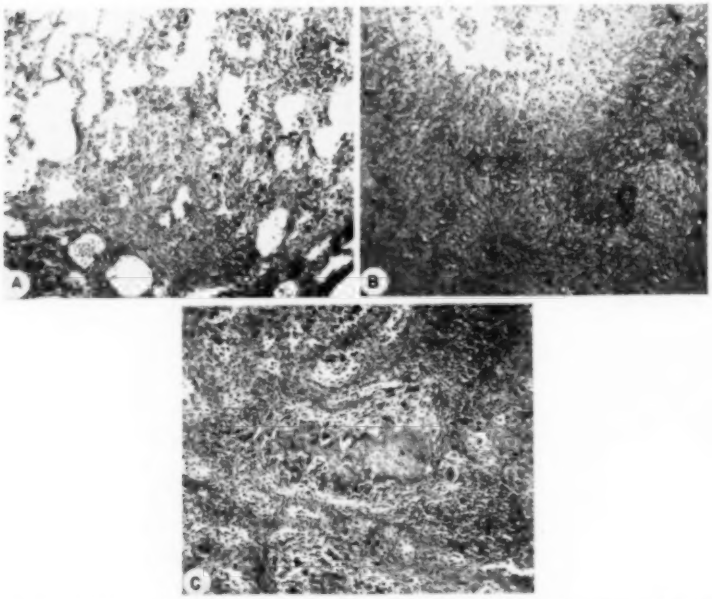

FIG. 7. Residual lesions after 300 days of streptomycin treatment in guinea pigs vaccinated one year prior to infection; from the 3 animals demonstrating unusual cystlike lesions.

A. No. A92 Lung. An irregular focus of scar tissue with slight associated lymphocytic infiltration, but without epithelioid cells, giant cells, or caseation. (100×)

B. No. A92 Hepatic lymph node, showing densely encapsulated caseous focus. Some of the caseous material had partially liquefied and was lost when the tissue was cut. (100×)

C. No. A90 Spleen. In the center of the field is a focus of caseous material surrounded by a delicate fibrous capsule, showing lymphocytic infiltration. The margins of the caseous material are irregular, with papilla-like projections of granulation tissue extending into it. (100×)

certain areas the epithelium was ulcerated, with well-developed epithelioid and caseous tubercles adjacent to the ulcerations (figure 6).

The spleen of one animal (No. A92) had many small nodular tubercles although the organ was not enlarged. Microscopically, this spleen revealed many large spreading epithelioid tubercles, some of which were partially encapsulated and some of which showed minute central necrosis. All of the animals revealed well-marked fibrous scars

around the Malpighian bodies, and one (No. A99) had two small foci of residual caseous material with delicate fibrous capsules, showing apparent invasion of the caseous area by granulation tissue (figure 7).

One animal (No. A92) showed enlargement of the iliac and inguinal nodes as well as a large and cystic hepatic node which, microscopically, was largely replaced by epithelioid tubercles with central caseation and peripheral scarring. The caseous material was seen to be liquefied in some areas (figure 7). The inguinal nodes of this animal revealed confluent epithelioid tubercles without caseation.

Typical gross and microscopic pictures seen in these 3 animals are presented in figures 5, 6, and 7.

Cultures were made directly from the purulent material of the cystic abscesses. In table 2 it is shown that cultures of 4 of the hepatic cysts were highly positive for tubercle bacilli and that in each case the recovered bacilli were susceptible to streptomycin *in vitro*.

Streptomycin Susceptibility of Recovered Tubercle Bacilli

With few exceptions, attempts were made to recover tubercle bacilli only when definite lesions were visible macroscopically.

A summary of the data regarding the drug sensitivity of tubercle bacilli recovered from the streptomycin-treated animals by culture or guinea pig subinoculation is presented in table 3.

In the first experiment (relapsed infections) cultures were made from the macerated organs of 3 guinea pigs which had been re-treated for 235 to 281 days. Only one was "positive," and this strain was susceptible to 2.5 γ per ml. Material from 2 of the 3 guinea pigs which survived the full 300 days of retreatment was cultured and subinoculated into normal guinea pigs. Both cultures were "negative," but tubercle bacilli were recovered from one animal by guinea pig subinoculation. This strain was also sensitive to 2.5 γ per ml.

Tubercle bacilli were recovered from 16 guinea pigs infected simultaneously with the animals of the first experiment and treated with streptomycin in the same manner for 90 days. These animals were then allowed to relapse and were not given any further treatment. Of the 18 strains of tubercle bacilli obtained from these 16 guinea pigs, 16 were sensitive to 0.5 γ of streptomycin per ml. of liquid medium, one was sensitive to 1.0 γ per ml., and one was sensitive to 2.5 γ per ml. (4).

Cultures were made from 4 vaccinated guinea pigs in the second experiment (vaccinated animals) which died after 32 to 180 days of streptomycin treatment; 2 were "negative" and 2, "positive." The 2 recovered strains were susceptible to 1.0 and 2.5 γ of streptomycin per ml., respectively. Five positive cultures were obtained from the 3 vaccinated guinea pigs which survived the full 300 days of streptomycin treatment. The recovered strains were all susceptible to streptomycin. Unfortunately, none of the cultures made from the lungs of these animals was positive for tubercle bacilli.

The H37Rv strain used to infect the animals was susceptible to 0.5 γ of streptomycin per ml. of Tween-albumin liquid medium. The greatest increase of

streptomycin resistance observed in the recovered strains, therefore, was approximately fourfold.

TABLE 3

SUMMARY OF DATA ON CULTURES FOR TUBERCLE BACILLI AND STREPTOMYCIN SUSCEPTIBILITY OF RECOVERED STRAINS

EXPERIMENT	ANIMAL NUMBER	DAYS OF STREPTOMYCIN TREATMENT	CULTURE	STREPTOMYCIN SUSCEPTIBILITY*	
				Direct, Solid Medium	Subculture Liquid Medium
Animals re-treated with streptomycin 165 days after a preliminary ninety-day course of streptomycin	370	235†	Positive	Susceptible	1.0–2.5‡
	402	259	Negative		
	467	281	Negative		
	687	300	Negative; guinea pig subinoculation, positive	Susceptible	1.0–2.5
	295	300	Negative; guinea pig subinoculation, also negative		
Vaccinated twelve months before infection	A97	148	Negative		
	663	155	Negative		
	383	180	Positive	Susceptible	1.0–2.5
	A99	300	Lungs, negative;		
			liver cyst No. 1, positive;	Susceptible	Not tested
			liver cyst No. 2, positive	Susceptible	Not tested
	A92	300	Lungs, negative;		
			liver cyst, positive;	Susceptible	Not tested
			spleen, positive	Susceptible	0.5–1.0
	A95	300	Lungs, negative;		
			liver cyst, positive	Susceptible	Not tested
	A93	300	Lungs, negative		
	A90	300	Lungs, negative		
Vaccinated one month before infection	A67	32	Positive	Susceptible	0.5–1.0
	A32	300	Abscess at site of infection, negative		

* Cultures were made directly on five tubes of ATS (egg-yolk, potato, glycerin) medium; two tubes without streptomycin and three with approximately 3.5, 15, and 200 γ per ml., respectively. Strains designated as susceptible showed no growth in the drug-containing tubes and heavy growth in the control tubes. When cultures produced less than 50 colonies in the control tubes, subcultures were made in Tween-albumin liquid medium and tested for susceptibility to streptomycin in the same liquid medium.

† Days of re-treatment for animals of first experiment.

‡ Resistant to 1.0 γ, sensitive to 2.5 γ, of streptomycin per ml. of medium.

DISCUSSION

In the first experiment, in which an attempt was made to produce chronic-type tuberculous lesions by allowing an infection in guinea pigs to relapse and progress slowly after a preliminary ninety-day course of streptomycin, an excel-

lent therapeutic response was obtained by re-treatment with streptomycin. Whereas all the control guinea pigs which were not given the benefit of a second course of streptomycin died within 336 days after the original course of drug therapy, none of those re-treated with streptomycin died of progressive tuberculosis. Of the 6 guinea pigs in the latter group which died prematurely of non-tuberculous infection, only one showed active spreading lesions, and 2 of the 3 animals which survived the full course of 300 days of re-treatment showed no evidence of tuberculous disease except tiny scars in some of the organs. Viable tubercle bacilli were recovered, however, by guinea pig inoculation from the third animal, which had several small nodular tubercles in the spleen and a small amount of caseation in the left iliac lymph node.

In the second experiment, the low native resistance of the guinea pigs was increased by previous vaccination and a much more desirable type of lesion, from the standpoint of the objects of the experiment, was produced. This was especially true in the group which had been vaccinated one year previous to infection, for the animals sacrificed just before treatment was started in the other members of the group showed pulmonary cavitation with great regularity, and the livers and spleens all demonstrated large discrete firm tubercles, in many of which the caseous material had liquefied to produce cystic abscesses.

The lesions in the liver and spleen called "cystic abscesses" were different from the cavities seen in the lungs. The former were solid lesions with caseous centers in which the caseous material had liquefied. When they were sectioned, the central purulent material escaped. The latter were mainly air-containing pulmonary cavities, although a few of these lesions near the surface of the lung were filled with serous fluid. Only one bronchial communication was definitely observed (figure 2), but no special effort was made to demonstrate such communications in other cavities. Because they were air-containing, however, it was assumed that most of the cavitary lesions in the lung did have a bronchial connection.

What was the effect of streptomycin on these chronic lesions? In general, a satisfactory therapeutic effect was noted although the uniformly excellent response seen in guinea pigs with acute tuberculosis was lacking. Three of the 7 animals vaccinated one year previous to infection which survived a treatment course of 300 days still presented large cystlike abscesses filled with creamy pus containing many tubercle bacilli, or large firm nodular tubercles, in some of the organs. The other 4 animals in this group showed nothing but small residual lesions or scars. It cannot be stated with any degree of certainty why some chronic lesions in certain animals disappeared entirely or left nothing but fibrous scars; whereas, in others, presumably the same type of lesions remained and even progressed during streptomycin therapy. Evidence that the variation in results was not entirely a result of the native general resistance of a particular animal is demonstrated by the fact that some guinea pigs, such as No. A99, which had large cystic abscesses in the liver, had excellent healing in the lungs and spleen. Moreover, animal No. A95, which had the largest caseous abscesses and cavities in the lungs, showed nothing but scars in the spleen and no evidence whatso-

ever of disease in the lymph nodes. The differing results cannot be ascribed to the emergence of drug-resistant organisms in certain lesions because all strains of tubercle bacilli isolated from many different cystic lesions were still drug susceptible.

These experiments have demonstrated that in the guinea pig many necrotic lesions may disappear during prolonged drug therapy, leaving only small residual scars. Whether necrotic lesions in the lungs disappear solely by sloughing and emptying into a bronchus or by slow absorption is a question which may only be answered by further experimentation.

The animals of *Group 2* in the second experiment (vaccinated one month previous to infection) demonstrated much less tuberculous disease at the pretreatment sacrifice period than those vaccinated the previous year, and the treated animals achieved a uniformly excellent response to streptomycin. This clearly demonstrates that the animals in *Group 2* had a significantly greater degree of protection against the virulent infection. Since both groups of animals were vaccinated with identical amounts of the same human attenuated strain, H37Ra, it may also be stated that the resistance afforded by such vaccination had decreased significantly during the year which had elapsed between the vaccination and the virulent infection.

The cutaneous sensitivity to 5 per cent Old Tuberculin also decreased during this time. Three weeks after vaccination, most of the animals in *Group 1* reacted strongly, producing areas of erythema and induration of approximately 18 mm. in diameter, with central necrosis. The intensity of the reactions began to fade after five to six months, and the final tuberculin reaction, studied just before infection with the virulent organisms, produced average areas of erythema and induration of approximately 10 mm. in diameter, without evidence of necrosis. A few of the guinea pigs showed doubtful reactions of only 5 by 5 mm., and one animal failed to react to tuberculoprotein.

In these experiments, the type of disease present at the start of treatment was determined by examining the organs of a representative sample of animals in each group. It was assumed that the remaining guinea pigs harbored similar lesions. A method which definitely established the existence of certain types of lesions in each animal before treatment was begun would eliminate the necessity of such an assumption. Further investigation of the effect of antimicrobial therapy on chronic necrotic lesions is being pursued in this laboratory, using the rabbit as the experimental animal. The animals are infected by inhalation and followed by serial roentgenograms of the lungs until shadows appear which are characteristic of large discrete caseous lesions and pulmonary cavity formation. At this time treatment is begun. It is hoped that such studies will help to solve some of the perplexing pathologic and bacteriologic problems encountered in the drug treatment of chronic caseous and cavitary tuberculosis in man.

SUMMARY

An attempt has been made in two experiments to produce chronic discrete tuberculous lesions in guinea pigs; first, by allowing an experimental tuberculous

infection to relapse after a preliminary ninety-day course of streptomycin; and second, by increasing the native resistance of the animals by vaccination with human attenuated tubercle bacilli previous to infection with virulent microorganisms. Streptomycin was then administered to the animals with these chronic lesions for a period of 300 days.

Particular attention was devoted to the questions of whether the chronic necrotic lesions would respond as well as the early acute lesions in guinea pigs; and whether streptomycin-resistant strains of tubercle bacilli could be recovered from such lesions after prolonged therapy.

Many of the necrotic lesions healed, leaving only residual scars. In certain animals, however, large cystlike abscesses filled with purulent material containing many viable tubercle bacilli were seen in the lungs, livers, and spleens. These occurred mainly in guinea pigs which had the most advanced necrotic lesions before treatment was started, in the group vaccinated one year previous to infection. Thus, it was found that chronic necrotic tuberculous lesions in the guinea pig responded to streptomycin treatment less favorably than did the acute type of disease.

Attempts were made to recover tubercle bacilli from 15 treated guinea pigs. A total of 9 cultures of *M. tuberculosi* was obtained from 7 of these animals. None of the 9 strains exhibited an appreciable degree of streptomycin resistance.

SUMARIO

La Estreptomicina en las Lesiones Tuberculosas Crónicas Discretas del Cobayo

En dos experimentos, tratóse de producir lesiones tuberculosas discretas crónicas en cobayos: primero, dejando que recidivara un grupo de animales infectados tras una tanda preliminar de noventa días de estreptomicina, y segundo, acrecentando la resistencia natural de los cobayos mediante la vacunación con bacilos tuberculosos humanos atenuados antes de infectarlos con microbios virulentos. Luego se administró estreptomicina a esos animales con lesiones crónicas durante un período de 300 días. Los problemas por resolver eran: (1) ¿Responderían las lesiones esfaceladas crónicas tan bien como las agudas incipientes en los cobayos?; y (2) después de una terapéutica prolongada ¿se obtendrían de dichas lesiones cepas estreptomicinorresistentes de bacilos tuberculosos?

Muchas de las lesiones esfaceladas sanaron, dejando únicamente cicatrices residuales. No obstante, en ciertos animales, obsevároнse en los pulmones, hígados y bazos grandes abscesos quistoideos llenos de materias purulentas que contenían muchos bacilos tuberculosos viables. Esto aconteció principalmente en los cobayos que tenían las más avanzadas lesiones necróticas antes de iniciar el tratamiento, en el grupo vacunado un año antes de la infección. Se descubrió, pues, que las lesiones tuberculosas esfaceladas crónicas del cobayo respondían menos favorablemente que las agudas a la estreptomicinoterapia.

Tratóse de recuperar bacilos tuberculosos de 15 cobayos tratados, obteniéndose un total de 9 cultivos positivos de 7 de dichos animales. Ninguna de las 9 cepas mostró estreptomicinorresistencia apreciable.

RESUME

La streptomycine chez le cobaye présentant des lésions tuberculeuses chroniques discrètes

Deux expérimentations ont été tentées dans le but de produire des lésions tuberculeuses chroniques discrètes chez le cobaye: primo, en permettant qu'une rechute se manifeste après un traitement préliminaire de quatre-vingt-dix jours avec la streptomycine; secondo,

en augmentant la résistance innée du cobaye par la vaccination avec des bacilles tuberculeux humains atténués, avant de procéder à l'inoculation avec des bacilles virulents. La streptomycine fut alors administrée à l'animal présentant ces lésions chroniques pendant une période de 300 jours. L'expérimentation chez le cobaye devait fournir une réponse aux questions suivantes: 1° Les lésions chroniques nécrotiques répondront-elles aussi bien que les lésions initiales aiguës? 2° Des souches de bacille tuberculeux résistantes seront-elles recouvrées à partir de telles lésions après un traitement prolongé?

Beaucoup de ces lésions nécrotiques se sont guéries, ne laissant que des séquelles cicatricielles. Cependant, chez certains animaux, on a observé dans les poumons, le foie et la rate, la présence de gros abcès remplis de matière purulente contenant de nombreux bacilles tuberculeux. Ces abcès se manifestaient principalement chez les cobayes dont les lésions nécrotiques étaient les plus avancées au moment où la thérapie a été instituée, dans le groupe vacciné une année avant l'inoculation. Dans ces conditions, on a constaté que les lésions tuberculeuses chroniques nécrotiques du cobaye répondaient moins bien à la streptomycinothérapie que les formes aiguës de l'affection.

Des tentatives ont été faites dans le but de recueillir des bacilles tuberculeux chez 15 cobayes traités. Au total, 9 cultures positives ont été obtenues à partir de 7 de ces animaux. Aucune des 9 souches n'a montré un degré de streptomycino-résistance appréciable.

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IRREGULAR DISCHARGE[1]

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INTRODUCTION

A major problem in all tuberculosis hospitals is the rate of discharge before patients are considered ready for return to the community. Tollen's classic monograph on the subject of "Irregular Discharge" (1) shows the enormity of the problem, highlighted by 55.6 per cent of such discharges among more than 6,000 total tuberculosis discharges from July, 1946 through June, 1947 in the Veterans Administration facilities. Tollen defined *irregular discharge* as "any termination of a living patient which is not medically sanctioned by professional authority." This included discharges designated A.W.O.L. (absent without leave) and Disciplinary Discharge. He discussed the peculiar social and psychologic implications of tuberculosis and the tuberculous patient. Among these were the necessity for long hospitalization and disruption from the community; lack of a specific cure; role of fear in a dreaded disease; and prevention of discharge of emotional and psychic energy.

Tollen reduced many reasons for irregular discharge given by professional staffs, medical social workers, and patients themselves to three factors: pressure factors outside the hospital—personal, social, economic; factors within the hospital—lack of adaptation, environment, attitude of personnel, regulations, and treatment; and inadequacies or weaknesses in personality of patients. Social workers placed almost equal emphasis on all factors, favoring the last, while the patients studied were not enthusiastic about the part personality deficiencies played but pointed to factors outside or inside the hospital.

Preventive measures mentioned by Tollen included intensive social case work prior to and during hospitalization; psychiatric care; more personalized treatment; more careful application of rules, ward arrangements, privileges, including passes; more varied recreation and rehabilitation; consideration of further financial aid for families of the tuberculous; and compulsory hospitalization of "open" cases of tuberculosis.

Because of the continuing importance of this problem, a study has been made of the reason for discharge of patients from the Tuberculosis Division, Baltimore City Hospitals, during the period 1946 through 1951.

OBSERVATIONS

The results of the analysis of the cause for discharge of the tuberculous patients during the six-year period, 1946 through 1951, may be seen in table 1.

Detailed study of the 25 (20 per cent) irregular discharges during 1951 revealed the following:

[1]From the Tuberculosis Division, Baltimore City Hospitals, Baltimore, Maryland.

There were 10 white females, 6 white males, 5 Negro females, and 4 Negro males in the Irregular Discharge Group. The hospital had approximately 50 per cent white and Negro, male and female, among 210 to 240 patients.

There were 11 far advanced cases, 10 moderately advanced, 3 minimal, and one pleural effusion.

There was a finding of tubercle bacilli in the sputum within six months in 13 cases; the sputum had been negative for tubercle bacilli for at least six months in 10 cases; in 2 patients the studies were insufficient for classification. The sputum findings included, in most instances, both sputum culture and culture of gastric aspirations.

The average hospitalization of the Irregular Discharge Group was 236 days and ranged from 4 to 988 days. Sixty-six per cent (15 patients) were in the hospital less than six months. At least 4 patients gave histories of chronic alcoholism. Only 2 had been previously hospitalized for tuberculosis.

TABLE 1

ANALYSIS OF THE CAUSE FOR DISCHARGE OF THE TUBERCULOUS PATIENTS DURING THE SIX-YEAR PERIOD 1946–1951

YEAR	DEATHS	TOTAL DISCHARGES	REGULAR DISCHARGES	IRREGULAR DISCHARGES	PER CENT OF IRREGULAR DISCHARGES
1946	165	173	80	93	56
1947	146	191	65	126	66
1948	118	143	77	41	46
1949	80	142	107	35	25
1950	84	118	104	14	12
1951	60	124	99	25	20

Evaluation of the reasons for irregular discharge was made from case histories, statement of the patient at time of discharge, medical social worker's comments, and statements of patient and public health nurse at follow-up visit when patient could be found. Many times several reasons were given; often the patient's reasons differed from the observations of the hospital or public health nurse. Using Tollen's classification, the major reasons were charted as follows:

Domestic or family problems	8
Immaturity or irresponsibility of the patient	7
Failure to comprehend seriousness of physical condition	2
Fear of surgery	1
Belief that proper care could be obtained at home	3
Desire to go to a hospital closer to home or to another hospital	4

Among the patients with domestic or family problems was a young Negro female who said she was told she would come in merely for thoracentesis; she was in her ninth pregnancy and had children and a drunken husband at home. An elderly white female had a serious family problem involving theft, but believed she had already been cured by prayer; when visited later she had sought no further medical care. One young Negro woman was determined to find her errant husband and take care of her four children. Another young white female had four children and had just married another man who had to

care for the children. An older white female left to be with a younger man, not her husband. There were usually problems which the patient did not discuss completely, and reasons could only be pieced together by observation and various extra channels.

Four patients with a long history of alcoholism were among those irregular discharges due to immaturity or irresponsibility of the patient. The others gave evidence during their hospital stay of inability to adapt to the hospital routine.

One young white female was a mild schizophrenic, but the psychiatric consultant believed that she should be kept in the tuberculosis hospital as long as possible. Another young white female who had recently had a baby could not be made to realize that she had tuberculosis. A minister told the patient and her physician that she had been cured by prayer and could leave the hospital.

A young Negro female had had a previous lobectomy. When told she needed further surgery, she walked out although she was told she could remain even if she decided against the surgical procedure.

Three patients decided they could receive proper care at home. One Negro female, just after surgery, had an additional personal problem she would not discuss. Another young white female had a problem with her husband and had a sister who had just been discharged to a satisfactory home. An elderly white female believed she could get along well at home after almost three years of hospitalization, although the home condition was unsatisfactory and the patient refused to have sputum (gastric) washings examined for tubercle bacilli.

Four patients gave as their reasons for leaving the desire to go to hospitals near home in another state or desire to have surgery elsewhere. Again, these reasons were not easily checked, and in one instance an older Negro male went to Washington, D. C., then to Henryton State Hospital, Maryland, where even now he refuses to allow the surgical procedure which has been recommended.

Several factors may have had important roles in the rapid decrease in irregular discharges at Baltimore City Hospitals since 1948. The state of Maryland changed regulations so that the "means" test for tuberculosis patients was abolished. A terrific economic burden was lifted from many patients who might otherwise have been unable to remain in the hospital while meager holdings were dissipated. Advances in treatment, particularly the use of streptomycin alone or with surgery, gave more patients hope of eventual discharge as arrested cases.

Finally, the hospital staff became more active in working as a team. Since 1949, weekly conferences have been held and have been attended by physicians, chief nurses, rehabilitation workers, the medical social worker, dietitian, chaplain, and various interested or invited guests. The newly admitted patients, the pending discharges, mutual problems, and plans have all been discussed and many long-existing difficulties have been satisfactorily settled. By pooling knowledge and resources, it has been possible to gain greater knowledge and understanding of the patients.

Monthly Pending Discharge Conferences have been established, at which the hospital staff meets with local agencies, including the health department and

department of public welfare. As many as 50 public health nurses at a time have discussed patients they knew at home before hospitalization and have given information of their background and problems. Representatives of all interested agencies and resources have been invited to discuss the entire question of discharge and rehabilitation. In many instances information obtained has helped other patients remain in the hospital or given opportunities to afford earlier discharge to a satisfactory home and economic environment.

Most of the medical social work has been done by one part-time, trained worker, who worked primarily with pending discharges. However, by wise utilization of nurses' and community agencies, she has worked with maximum efficiency.

It is difficult to establish how many of the regular discharges at one time or another were ready to leave against advice. It is known that many of the patients originally came into the hospital prepared to stay several weeks or months, as predicted by an outside physician. Careful explanation and handling by the staff kept most of these through a year or two and eventually to planned discharge. Others became dissatisfied with food, regulations, staff, other patients, treatments, or had numerous minor complaints that threatened to make them leave. The treatment of tuberculosis is long and tiresome, and the present writer believes that at least 75 per cent and perhaps all patients at one time or another reach a point at which they are ready to leave the hospital against medical advice. Some are too sick to carry out this decision, most realize the impracticality of such a plan, and it is the minority of patients who do leave against the advice of the physicians.

DISCUSSION

Attempts to reduce irregular discharges from tuberculosis hospitals should begin with a careful analysis of reasons for occurrence. Next must come a decision in regard to which reasons are preventable. Finally, a detailed attack can be outlined.

Each case presents separate individual problems, but in the over-all picture certain facts appear so consistently as to allow reasonable generalization. Minimal cases of pulmonary tuberculosis need shorter hospitalization. Early diagnosis should find more minimal cases. These patients with favorable outlook have more reason to remain hospitalized until ready to be discharged to the community.

Complete evaluation of the patient, including emotional, social, and economic status, should be done as soon as possible. A careful history will often allow recognition of problems, not only acute but incipient. If all outside problems could be settled before hospitalization, the path to recovery would be smoother. Many women consider proper care and placement of children their chief problem. Men frequently need help with financial aid for their families. Such an evaluation comprised mostly of social case-work type of investigation needs to be continuous throughout hospitalization. Periodic discussion with families and friends may permit problems to be solved before they become major issues.

Once problems become critical, many patients lose their perspective and the ability to reason calmly, and leave the hospital as their only course.

Education and orientation concerning tuberculosis and the patient's own disease process should be given early. Often the first information given makes the greatest impression and, if it is faulty or incomplete, the patient does not have a true appreciation of his condition. A basis for the future should be laid in discussion at the time of presentation of the original diagnosis. Too often does the patient believe he will be hospitalized only a few weeks and makes plans accordingly, only to be shocked at his hospital physician's estimate.

Once in the hospital there should be a continuous educational program, including orientation, discussion of his disease with treatment plan, hospital regulations and routines, and sources of help. A real attempt should be made to show the patient he is in a hospital particularly suited to treat tuberculosis, where everything will be done to return him well to the community as soon as possible. Each member of the hospital staff plays a role. Physician, nurse, minister, medical social worker, occupational therapist, rehabilitation workers, librarian, dietitian, all come in contact with the patient and on their coordinated effort depends his physical and mental well-being. Frequent visits by all of these, particularly an understanding physician, can alleviate mounting emotional tension. As time goes on, changes in therapy or routine should be carefully explained.

Occasionally, emergency problems arise or a crisis occurs in a smouldering problem. If all available agencies cannot handle the problem, a short "emergency" pass may be the solution. The patient must be in sufficiently good condition to allow this, and hospital staff assistance is usually added. Often such problems are considered personal and private. Patients may refuse help and leave the hospital to handle the situation their own way. Close association and understanding between the staff and patients should keep such occurrences to a minimum. Once a patient leaves the hospital against advice, even for emergency situations, it is unlikely he will return immediately for continuation of treatment. In a few cases in the present series, attempts were made to have such patients seen by staff or public health nurses and urged to return within a few days. In rare instances, this has been successful and the practice well worth the effort.

Psychiatric care is valuable, at times mandatory, for treatment or commitment of psychotic patients. Mild schizophrenic or depressed patients may offer considerable problems. Some patients have home conditions which allow return earlier than others. Full use should be made of such opportunities, but only after careful consultation with the family and physician or clinic which will maintain the follow-up observation.

There are a group of ambulant chronic patients, healthy in other respects but who discharge tubercle bacilli in the sputum intermittently. They are considered to have received "maximum benefit of hospitalization" although they are still a danger to the community. Every attempt should be made to evaluate their home and community situation. Many can be placed in the

community as a minimal public health hazard and as useful citizens. Some patients are content to relax in institutions, getting food, lodging, and what they consider an adequate existence. Others think that institutional life is too restricted or inadequate, have no community responsibility, and leave when an opportunity presents itself. Others have homes available but cannot be considered safe risks.

At the present time the solution to the chronic "positive" ambulant patient who leaves an institution against advice seems to be compulsory hospitalization. Some day a carefully planned rehabilitation facility will find work and compensation for these unfortunates under living conditions resembling those in the healthy community.

This problem of irregular discharges is constantly with us. Solution depends on vigilant, well-organized, efficient, and understanding staff work by all members of the tuberculosis team, hospital, and community.

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THE REVERSAL OF THE ACTIVITY OF ANTITUBERCULOUS COMPOUNDS IN VITRO[1]

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INTRODUCTION

Investigators in the field of chemotherapy often are disappointed by the failure *in vivo* of a compound which has shown great promise against infectious agents *in vitro*. This has been the case since the days of Ehrlich's work on the arsenicals.

In 1932, Wells and Long (5) attributed such failures to changes which a specific chemotherapeutic agent may undergo in the animal tissues, the binding and inactivating power of the plasma, and the effect of the growing bacilli on the drug. D'Arcy Hart (1), in reviewing the status of the chemotherapy of tuberculosis in 1946, stated that the ultimate failure of a drug which is active *in vitro* could be caused by its failure to reach the site of infection, its inactivation by antagonistic substances in the body, or by the excessive speed of its metabolic destruction or excretion.

In a thorough and systematic study of a drug's activity *in vivo*, consideration should be given to the rate of its absorption into the blood stream, its distribution in the body, and its rate of destruction and excretion. A further consideration is the concentration of drug in the blood as compared with its inhibiting level *in vitro*. An additional factor is the possible interfering action of blood and other body constituents on the activity of the compound.

It is evident that many factors play roles in causing differences between *in vitro* and *in vivo* activities of various compounds. In large chemotherapeutic programs involving the study of several thousand compounds, it would be a hopeless task to attempt to examine each of these factors with every compound studied. Hence, efforts to lay down general principles applicable to specific types of compounds would appear to be worth while.

In a recent publication by Donovick, Rake, and Titus (2), it was shown that, even though factors such as absorption, blood level, and serum inactivation were accounted for, *in vitro* and *in vivo* activities were not easily correlated. It was of interest, therefore, to investigate more thoroughly other factors which might play a role *in vivo* in reducing or destroying the antituberculous action of compounds showing high activity *in vitro*. It seemed reasonable to assume that metabolite-antimetabolite competition in the body could explain the failure of many of these compounds.

Woods and Fildes (6), in their studies on the inactivation of sulfonamides, demonstrated that knowledge of the point of interference of antimetabolites could yield new information on the essential metabolism of the microorganism being studied. The metabolism of *Mycobacterium tuberculosis* presents a somewhat

[1] From Squibb Institute for Medical Research, New Brunswick, New Jersey.

special and intriguing problem. For growth *in vitro*, the demands of *M. tuberculosis* are remarkably simple. In a medium containing potassium, magnesium, sulfate, and phosphate ions, together with an inorganic ammonium salt as a nitrogen source, and a simple source of carbon such as glycerol, growth occurs readily for many strains. If, in addition, a surface active agent is added, such as Tween 80[2] or Triton A-20[3] at low concentrations, the rate of growth may be enhanced. Higher concentrations of these latter compounds are inhibitory. Thus, from such simple components in the culture medium this microorganism, which has at times been thought to be fastidious in its demands, is capable of synthesizing complex proteins, enzymes, and many growth factors.

The microorganism used in the present studies was *M. tuberculosis* var. *bovis*, strain BCG. Although the value of using the nonpathogenic BGC strain in an *in vitro* test for antituberculous compounds might be questioned, comparative tests of the *in vitro* activities of many compounds showed that the BCG, Ravenel, and H37Rv strains of *M. tuberculosis* were remarkably similar in their *in vitro* sensitivities, and it appeared reasonable to use the response of BCG as a measure of the response of the pathogenic strain under the conditions of these tests.

METHODS

Briefly, the reversal studies were set up in the following manner. The compound being studied was added to 18 ml. of a modified Kirchner's medium, shown in table 1, to give a final concentration approximately twice the minimal inhibiting level. The reversing agent was added to the same flask, and the flask was then inoculated with a standard amount of culture. After seven days' incubation at 37°C., the turbidity of the flask was measured by means of a photoelectric colorimeter and compared with control flasks containing drug alone and with flasks with no drug. For the purpose of comparing results of different experiments, the coefficient of inactivation was calculated, using the equation shown in table 2. Coefficients below 25 were not considered significant reversal of activity.

OBSERVATIONS

An approach which has been used in designing compounds potentially of chemotherapeutic use has been the preparation of an analogue of a compound known to be a metabolite for the particular pathogenic organism of interest. As has already been mentioned, *M. tuberculosis* does not readily lend itself to such an approach because of its simple growth requirements. There was available in these laboratories, however, a very large supply of compounds which caused inhibition of the growth of BCG *in vitro* at relatively low concentrations (0.03 mg. per ml. and less). Within this wide selection of compounds it appeared that some might possibly be reversed by certain compounds which were known to be essential nutrients for other microorganisms, but not known to be such for *M. tuberculosis*. The basis for such a tentative assumption was that the chemical or

[2] Proprietary name for Sorbitan monooleate, obtained from the Atlas Powder Co., Wilmington, Delaware.

[3] Proprietary name for aryl-alkyl polyether of phenol, obtained from Rohm and Haas, Philadelphia, Pa.

physical structure of some of these antituberculous compounds resembled those of some of the known essential metabolites. It was soon apparent that this approach was not fruitful, and a broader empirical method was attempted. Large groups of compounds were screened by testing them in the presence of the following: (*1*) serum, (*2*) vitamin mixtures, (*3*) para-aminobenzoic acid (PABA), (*4*) casein hydrolysate, and (*5*) pure amino acid mixtures.

Serum was used essentially for eliminating compounds at least temporarily from further study, since it was felt that elucidation of the significance of reversal by this substance could in itself be a very long-term study. Some of these findings with serum have been presented in an earlier paper (2). It might be

TABLE 1

KIRCHNER'S MEDIUM WITH TRITON A-20

	PER CENT
Asparagine	0.5
Glycerol	2.0
Sodium citrate	0.25
$MgSO_4 \cdot 7H_2O$	0.06
Na_2HPO_4	0.3
KH_2PO_4	0.4
Triton A-20*	0.02

* Described by the manufacturer, Rohm & Haas Company, Philadelphia, Pennsylvania, as an aryl-alkyl polyether of phenol.

TABLE 2

CALCULATION OF PER CENT REVERSAL OF DRUG ACTIVITY

$$\frac{\log D - \log (D + I)}{\log D - \log C} \times 100 = \text{per cent reversal}$$

where: D = per cent transmission with drug alone
D + I = per cent transmission with drug + inactivator
C = per cent transmission of culture alone

added in passing that serum albumin appeared to be responsible for much of the reversing ability of serum, while serum globulin apparently had little effect.

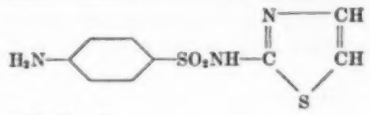
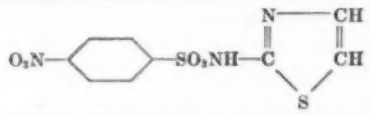
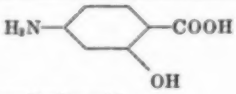
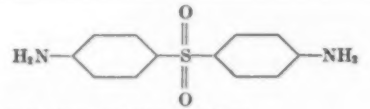
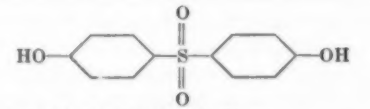
The vitamin mixtures were made up roughly on the basis of the vitamin composition which has been reported in the literature to be found in *M. tuberculosis*. Para-aminobenzoic acid was not included in these mixtures, since this compound was investigated in special studies. The activity of very few compounds was affected by the vitamin mixtures employed.

Para-aminobenzoic acid, of course, has received wide attention, but generally in connection with the metabolism of organisms other than *M. tuberculosis*. Nevertheless, Goodacre and associates (4) have shown that PABA can reduce the activity of *p*-aminosalicylic acid; and, similarly, Fitzgerald and Feinstone (3) have reported that PABA can reduce the activity of certain sulfonamides for acid-fast bacteria. Hence, there was already good reason to believe that

PABA played a role in the metabolism of *M. tuberculosis* similar to that in other types of microorganisms.

TABLE 3

REVERSAL BY *p*-AMINOBENZOIC ACID (PABA)

INHIBITOR		PER CENT REVERSAL BY PABA (0.1 MG/ML)
Structure	Mg. per Ml.	
 sulfathiazole	0.05	79
 *p*-nitro-N-2-thiazolylbenzene-sulfonamide	0.01	6
 *p*-aminosalicylic acid	0.001	86
p-nitrobenzoic acid	0.06	5
 *p,p'*-diaminodiphenylsulfone	0.05	78
 *p,p'*-dihydroxydiphenylsulfone	0.1	0

Reversals were calculated by the method shown in table 2.

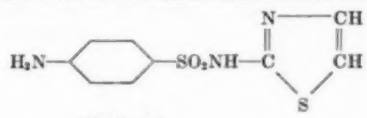
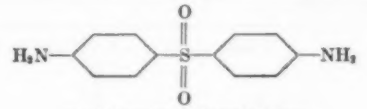
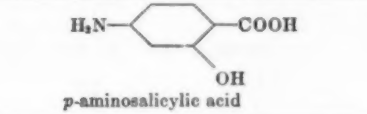
For the studies with PABA, 100 compounds were selected on the basis of high *in vitro* activity, regardless of their activity *in vivo;* none of the compounds were seriously affected by the presence of serum. Of these, less than 10 per cent were inactivated by PABA to a significant degree. Of the drugs affected by

PABA, there was more than 75 per cent inactivation of sulfathiazole, diaminodiphenylsulfone, and para-aminosalicylic acid. A comparison of the ability of PABA to reverse several closely related compounds is shown in table 3. It is of interest that PABA had no effect on the para nitro analogue of sulfathiazole, on paranitrobenzoic acid, or on dihydroxydiphenylsulfone.

As can be seen in table 4, the reversal of the activity of sulfathiazole and diaminodiphenylsulfone by PABA is clearly competitive, a twofold drug increase reducing this action appreciably. It appears, however, that the action of PABA on para-aminosalicylic acid is of a different nature, for even at a thousandfold increase in the concentration of this drug PABA still caused reversal.

TABLE 4

EFFECT OF INHIBITOR CONCENTRATION ON REVERSAL BY *p*-AMINOBENZOIC ACID (PABA)

INHIBITOR		INHIBITOR/PABA MOLAR RATIO	PER CENT REVERSAL BY PABA (0.1 MG/ML)
Structure	Mg. per Ml.		
 sulfathiazole	0.1	0.5	78
	0.2	1	49
	0.4	2	13
	0.8	4	5
 *p,p'*-diaminodiphenylsulfone	0.05	0.25	81
	0.1	0.5	76
	0.2	1	53
	0.4	2	0
 *p*-aminosalicylic acid	0.0002	0.002	100
	0.002	0.02	93
	0.02	0.2	94
	0.2	2	86

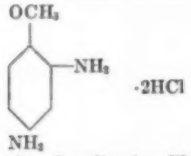
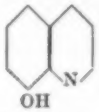
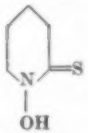
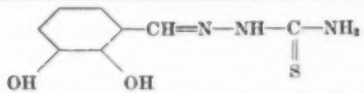
It was not possible to demonstrate any reversing action by folic acid of any compounds reversed by PABA. Such activity would be expected if one assumed that the drugs compete with PABA and block the synthesis of folic acid. It is possible that other folic acid-containing compounds may effect such reversals, but in the absence of positive evidence the present writers believe that this remains an open question.

On the basis of reports on the inhibition of microorganisms by amino acid analogues, amino acids were also considered as possible inactivating agents. An enzymatic hydrolysate of casein, chosen as a mixture of naturally occurring amino acids, was used in reversal studies on more than 100 compounds active *in vitro*. Approximately 40 per cent of these compounds were appreciably reduced in activity in the presence of casein hydrolysate.

When these results were compared with the reversals of these compounds by serum and by PABA, it was apparent that in most instances the three agents acted on different compounds. Considering the heterogenous nature of the drugs

TABLE 5

REVERSAL BY AMINO ACIDS

INHIBITOR		PER CENT REVERSAL OF ACTIVITY BY		
Structure	Mg. per Ml.	Casein Hydrolysate	Amino Acid Mixture	Specific Amino Acids
2,6-diamino-3-iodopyridine	0.01	73	72	Methionine 67
 4-methoxy-*m*-phenylenediamine dihydrochloride	0.02	80	100	Lysine 100 Proline 100
 8-hydroxyquinoline	0.0005	78	77	Histidine 80
 N-hydroxy-2-pyridinethione	0.00001	83	57	Histidine 83
 2,3-dihydroxybenzaldehyde, 3-thiosemicarbazone	0.00025	82	66	Histidine 50

Reversals were calculated by the method shown in table 2.

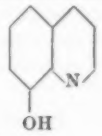
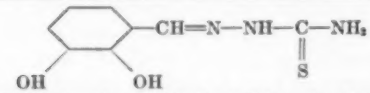
selected for this study, it was interesting to note how frequently compounds of similar structure fell into similar groups in this comparison.

A mixture of nineteen pure amino acids was then prepared to approximate the amino acid composition of casein. The reversing ability of this mixture was

almost identical to that of casein hydrolysate. The amino acids were then divided into four groups, and the various compounds were tested for their activity in the presence of these separate amino acid groups. This was done in order to simplify the problem of detecting which amino acids were causing the reversal. When any group of amino acids was found to be capable of reducing the activity of a given compound, the individual amino acids in that group were then tested for this reversing ability. By this process of elimination, histidine was found to reverse the activity of 8-hydroxyquinoline, 2,3-dihydroxybenzaldehyde, 3-thio-

TABLE 6

REVERSAL BY HISTIDINE AND HEMATIN

INHIBITOR		PER CENT REVERSAL OF INHIBITOR BY	
Structure	Mg. per Ml.	Histidine 0.6 Mg. per Ml.	Hematin 0.005 Mg. per ml.
 8-hydroxyquinoline	0.0005	80	90
N-hydroxy-2-pyridinethione	0.00001	83	95
 2,3-dihydroxybenzaldehyde, 3-thiosemicarbazone	0.00025	50	84

Reversals were calculated by the method shown in table 2.

semicarbazone, and several pyridine-thiones. 2,6-Diamino-3-iodopyridine was inactivated by methionine, while lysine and proline were both capable of reversing 4-methoxy-*m*-phenylenediamine-dihydrochloride (table 5).

The nature of these reversals may provide a valuable clue with regard to the mode of action of antituberculous drugs. If the action of these drugs lies in the inhibition of amino acid synthesis, then the amino acid at a concentration sufficient to meet the needs of the tubercle bacillus should inactivate any concentration of drug. If, however, the drugs act by interfering with the utilization of a specific amino acid, then a competitive reversal exists, and the concentration of amino acid required for reversal is dependent on the drug concentration. With compounds reported here, the reversals by amino acids were of a competitive

nature, with a rather constant ratio being maintained between the concentration of drug and that of the amino acid required for significant reversal of activity.

The group of compounds reversed by histidine was particularly interesting because they were also found to be inactivated by hematin (table 6). Since 8-hydroxyquinoline is known to form complexes with metals, it seemed possible that the organism could substitute the iron in hematin for that bound by the drug. The role of histidine in such reversals might also be connected with iron complexing, since histidine is believed to be present in the porphyrin residue of cytochrome in coordination with the iron atom.

If these compounds are active solely because of their chelating ability, it should be possible to reverse their activities by addition of sufficient iron to replace that bound by the complex. Studies by the present investigators of reversal by iron have yielded variable and confusing results thus far, particularly with the pyridine-thione compounds. These investigations with iron as well as with other metals are being continued.

The present report is only an introduction to a most complex problem and is the first of a series planned to cover various aspects of this subject. Inactivation of compounds by blood components and elucidation of the components involved present a formidable problem in itself. Inactivation by simpler systems such as PABA and/or amino acids offers a more encouraging line of approach. It is interesting that some of the compounds showing the highest activity *in vitro* but failing *in vivo* are those reversed by histidine, and there are good indications that some of these are also inactivated by iron. This leads one back to the question of the intermediary metabolism of this rather remarkably bio-synthetic organism. It is in this direction that most intensive efforts are being directed at present in these laboratories.

SUMMARY

By the employment of BCG, grown in a simple synthetic medium as the test organism, it was possible to confirm the ability of *p*-aminobenzoic acid (PABA) to reverse the inhibition of growth by sulfathiazole, *p*-aminosalicylic acid, and *p,p'*-diaminodiphenylsulfone. The inhibition of BCG growth by *p*-nitrobenzoic acid, *p*-nitro-N-(2-thiazolyl) benzenesulfonamide, and *p,p'*-dihydroxydiphenylsulfone was not reversed by PABA.

A systematic study of the reversing effect of amino acids disclosed that lysine and proline reversed the effect of 4-methoxy-*m*-phenylenediaminedihydrochloride. Histidine reversed the effects of 1-hydroxy-2(1)-pyridinethione; 8-hydroxyquinoline; and 2,3-dihydroxybenzaldehyde, 3 thiosemicarbazone. Methionine reversed the action of 2,6-diamino, 3-iodopyridine. The activity of the compounds which were reversed by histidine could also be reversed by hematin.

SUMARIO

Viraje de la Actividad de los Compuestos Antituberculosos in Vitro

Empleando BCG, cultivado en un sencillo medio sintético, como microorganismo de ensayo, fué posible confirmar la capacidad del ácido *p*-aminobenzoico para revertir la inhibición del desarrollo por el sulfatiazol, el ácido *p*-aminosalicílico y la *p,p'*-diaminodi-

fenilsulfona. El PABA no hizo virar la inhibición del crecimiento del BCG producida por el ácido *p*-nitrobenzoico, la *p*-nitro-N-(2-tiazolil) bencenosulfonamida y la *p,p'*-dihidroxidifenilsulfona.

Un estudio sistemático del efecto revertidor de los ácidos amínicos reveló que la lisina y la prolina viraban el efecto del biclorhidrato de 4-metoxi-*m*-fenilenodiamina. La histidina invirtió los efectos de la 1-hidroxi-2(1)-piridina-tiona, la 8-hidroxiquinolina, y la 2,3-dihidroxibenzaldehido, 3 tiosemicarbazona. La metionina invirtió la acción de la 2,6-diamino, 3-yodopirina. Los compuestos revertidos por la histidina lo fueron también por la hematina.

RESUME

Inversion de l'action de composés antituberculeux, in vitro

Il a été possible, en employant comme microorganisme d'épreuve, le BCG cultivé dans un milieu synthétique simple, de confirmer le pouvoir que possède l'acide *p*-aminobenzoïque d'inverser l'inhibition de la culture par le sulfathiazol, l'acide *p*-aminosalicylique et le *p,p'*-diaminodiphénylsulfone. L'inhibition du développement du BCG par l'acide *p*-nitrobenzoïque, *p*-nitro-N-(2-thiazolyl) benzènesulfonamide et par le *p,p'*-dihydroxydiphénylsulfone n'était pas inversée par le PABA.

Une étude systématique de l'effet d'inversion des acides aminés a montré que la lyzine et la proline inversaient l'action du chlorhydrate de 4-méthoxy-*m*-phénylènediamine. L'histidine inversait les effets de 1-hydroxy-2(1)-pyridine-thione; de 8-hydroxyquinoline et de 2,3-dihydroxybenzaldéhyde, 3 thiosémicarbazone. La méthionine inversait l'action de la 2,6-diamino, 3-iodopyridine. Ces composés, sur lesquels l'histidine exerçait une action d'inversion, étaient également inversés par l'hématine.

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THE EFFECT OF PROBENECID (BENEMID) IN ENHANCING PARA-AMINOSALICYLIC ACID CONCENTRATIONS IN THE BLOOD[1,2]

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INTRODUCTION

The efficacy of para-aminosalicylic acid (PAS) alone or in combination with streptomycin in the treatment of tuberculosis is well established. Effective bacteriostatic levels against the tubercle bacillus are reported to be 0.015 to 1.5 mg. per 100 ml. of plasma (1). It is probable, however, that if such concentrations are to be achieved in the extravascular diseased areas of the body, even higher concentrations must be maintained in the plasma.

Para-aminosalicylic acid is readily absorbed from the gastrointestinal tract and rapidly excreted in the urine. Approximately 85 per cent of a given dose may be recovered from the urine as the conjugated or free amines in a period of seven hours (2). Plasma concentrations of PAS after a single dose of 4 gm. will be in the vicinity of 9.5 mg. per 100 ml. in one-half hour, 8 mg. per 100 ml. in two hours, 2 mg. per 100 ml. in four hours, and 0.5 mg. per 100 ml. in six hours (3). The rapidity of urinary excretion of PAS makes frequent large doses of the drug desirable to maintain high plasma concentrations, but the limiting factor is the fact that most patients cannot tolerate a daily dose in excess of 12 gm., due to gastrointestinal irritation. A drug capable of delaying the renal excretion of PAS and thus increasing blood concentrations with smaller doses of PAS would produce the same effect as larger doses of PAS alone. Probenecid (Benemid[3]) has been reported to have such potentialities.

Probenecid has been demonstrated to exert an inhibitory effect on the renal excretion of PAS with a concomitant rise in plasma concentrations of the drug (4). The mechanism in the renal excretion of PAS appears to involve the inhibition of an enzyme system active in combining PAS with glycine (5). Presumably, the conjugate is excreted much more readily than free PAS. Inhibition of this enzyme system, therefore, should result in higher and more sustained PAS concentrations. Plasma concentrations of PAS in patients who are also receiving probenecid have been shown to be elevated approximately two to four times the levels attained without probenecid (3, 6). Probenecid has proved to be a relatively nontoxic drug both in animals and in man (7, 8). The optimal dose has been determined to be 0.5 gm. every six hours orally for most patients.

[1] From the Veterans Administration Hospital and the University of Minnesota Medical School, Minneapolis, Minnesota.

[2] Published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

[3] Proprietary name for *p*-(di-*n*-propylsulfamyl)-benzoic acid, manufactured by Sharp & Dohme, Inc. The Benemid was supplied through the courtesy of Dr. W. P. Boger, Medical Director, Sharp & Dohme, Inc.

The possibility of employing smaller doses of PAS with probenecid and avoiding gastrointestinal symptoms noted with larger doses, yet maintaining blood concentrations approximating those attained on higher dosage, has been the question attacked in the present study.

METHOD OF STUDY

Fifteen tuberculous patients receiving oral doses of 12 gm. of PAS (as a solution of sodium PAS) daily, as well as intramuscular streptomycin, were studied. Whole blood concentrations of PAS were determined by the method of Way and his associates (2). Control values were determined on patients receiving (*A*) 4 gm. of PAS three times daily, and (*B*) 3 gm. of PAS four times daily. The concentrations were determined at three hours and four hours after the morning dose of PAS. Probenecid was then given in an oral dose

TABLE 1

COMPARISON OF THE EFFECT OF PROBENECID (BENEMID) ON MEAN BLOOD PAS CONCENTRATIONS IN VARIOUS DOSAGE SCHEDULES OF PAS

DOSAGE SCHEDULE	THREE HOURS		FOUR HOURS	
	Number of Samples	Mg. per 100 Ml.	Number of Samples	Mg. per 100 Ml.
PAS, 4 gm. three times daily				
Without probenecid	23	3.12 ± 0.99	20	2.35 ± 0.77
With probenecid*	21	4.76 ± 1.58†	27	2.62 ± 0.80
PAS, 3 gm. four times daily				
Without probenecid	24	2.93 ± 1.01	21	1.72 ± 0.61
With probenecid	28	4.15 ± 1.22†	35	2.53 ± 0.81†
PAS, 2 gm. four times daily				
With probenecid	26	2.16 ± 0.55	28	1.49 ± 0.58

* Probenecid dosage, 0.5 gm. four times daily in all series.

† Significant difference (P less than 0.01).

of 0.5 gm. four times daily, with the patients remaining on the same dosage of PAS. PAS blood concentrations were determined at weekly intervals thereafter for four weeks. The same patients were subsequently placed on a regimen of 2 gm. of PAS four times daily, with the same dosage of probenecid, and similarly studied.

RESULTS

Mean control values for blood PAS in *Group A* at three and four hours after the morning dose were 3.12 mg. per 100 ml. and 2.35 mg. per 100 ml., respectively. For *Group B* the concentrations in the controls were 2.93 mg. per 100 ml. and 1.72 mg. per 100 ml. at three and four hours, respectively (table 1). While on probenecid, the concentrations rose to 4.76 mg. per 100 ml. and 2.62 mg. per 100 ml. at three and four hours for *Group A*, and 4.15 mg. per 100 ml. and 2.53 mg. per 100 ml. for *Group B*. The differences at the three-hour concentration between the control values and those after the addition of probenecid are statistically significant for both *Groups A* and *B*. The difference in the concentrations at four hours is significant only for *Group B*. Comparison of these results by extent of increase in PAS concentrations (table 2) indicates an enhancement of 40 to 50 per cent by the addition of probenecid.

Mean concentrations for the patients receiving 8 gm. of PAS daily with probenecid, *Group C*, were 2.16 mg. per 100 ml. and 1.49 mg. per 100 ml. at three and four hours after the morning dose. The concentrations attained by

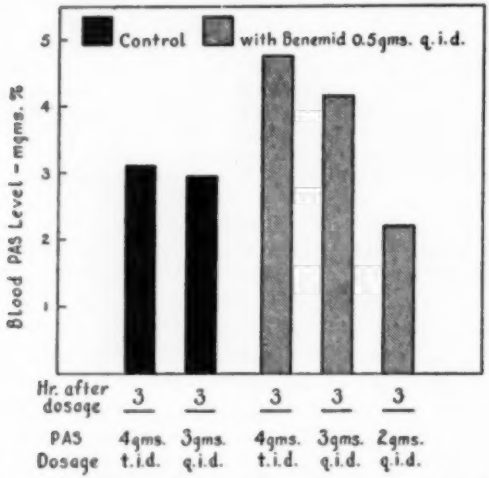

FIG. 1. Mean blood PAS concentrations with and without probenecid (Benemid)

TABLE 2

EFFECT OF PROBENECID (BENEMID) UPON INCREASING MEAN BLOOD PAS CONCENTRATIONS

DOSAGE SCHEDULE	THREE HOURS		FOUR HOURS	
	Number of Samples	Mg. per 100 Ml.	Number of Samples	Mg. per 100 Ml.
PAS, 4 gm. three times daily				
With probenecid*	21	4.76†	27	2.62
Without probenecid	23	3.12	20	2.35
Increase		1.64		0.27
Per cent of increase		52		11
PAS, 3 gm. four times daily				
With probenecid	28	4.15†	35	2.53†
Without probenecid	24	2.93	21	1.72
Increase		1.22		0.81
Per cent of increase		41		47

* Probenecid dosage; 0.5 gm. four times daily in all series.

† Significant difference (P less than 0.01).

this group are lower than control values for either of the 12 gm. PAS daily regimens without probenecid, but approximate the concentrations obtained with the regimen of 3 gm. four times daily. No evidences of toxicity attributable

to probenecid were noted in any of the patients receiving the drug. Hemograms and urinalyses remained unchanged. The increased blood concentrations of PAS with probenecid did not result in any evidences of toxicity from PAS.

DISCUSSION

The lack of knowledge concerning the minimal dosage necessary to maintain the optimal effect upon the rate of emergence of resistance of streptomycin-resistant tubercle bacilli makes the choice of the desirable regimen difficult. Studies by Warner (9) and Lehman (10) have suggested that a dose of 8 to 12 gm. of oral PAS daily, preferably the latter, gives the best results therapeutically, as well as having the greatest effect on the rate of emergence of resistance to streptomycin when PAS is given in combination with streptomycin. Carr and his associates (11) have reported a parallel bacteriostatic effect of increased plasma concentrations of PAS upon populations of tubercle bacilli *in vitro*, which suggests that the enhancement of the blood concentrations of PAS is advantageous. For patients unable to tolerate the 12 gm. daily dose of PAS, the results of the present study suggest that the use of an 8 gm. daily dose of PAS with probenecid would result in blood concentrations comparable with those obtained with the 12 gm. dose alone.

SUMMARY

Blood PAS concentrations of 15 tuberculous patients who received 12 gm. of oral PAS daily were studied before and after the addition of 2 gm. of probenecid (Benemid) daily to PAS dosage, resulting in a 40 to 50 per cent enhancement of blood PAS concentrations after the addition of probenecid.

Reduction of PAS dosage to 8 gm. daily, together with 2 gm. of probenecid, resulted in blood concentrations below those attained by either 12 gm. regimen, with or without probenecid, but were close to the concentrations obtained with 3 gm. of PAS four times a day.

No toxic effects of probenecid were observed.

It is suggested that patients who cannot tolerate a 12 gm. daily dosage of PAS may benefit by reducing the dose to 8 gm. daily and adding 2 gm. of probenecid daily.

SUMARIO

El Efecto del Probenecid (Benemid) en la Elevación de las Concentraciones Sanguíneas del Acido Para-Aminosalicílico

Las concentraciones sanguíneas de PAS en 15 tuberculosos que recibieron 12 gm. diarios de dicha droga por vía oral fueron estudiadas antes y después de agregar 2 gm. diarios de probenecid (Benemid) a la dosis de PAS, observándose una elevación de 40 a 50 por ciento en el tenor sanguíneo de PAS después de agregar el probenecid.

La reducción de la dosis de PAS a 8 gm. diarios, unidos a 2 gm. de probenecid, dió por resultado niveles sanguíneos inferiores a los obtenidos con uno u otro de los regímenes de 12 gm., con o sin probenecid, pero próximos a los conseguidos con 3 gm. de PAS cuatro veces diarias, solo.

No se observaron efectos tóxicos del probenecid.

Sugiérese que los enfermos que no pueden tolerar una dosis de 12 gm. diarios de PAS quizás se beneficien rebajando la dosis a 8 gm. diarios y agregando 2 gm. diarios de probenecid.

RESUME

L'effet de renforcement produit par le Probenicid (Benemid) sur la concentration sanguine de l'acide para-aminosalicylique

La concentration sanguine du PAS a été étudiée chez 15 tuberculeux recevant une dose quotidienne orale de 12 g. de PAS, avant et après l'adjonction de 2 g. de probenecid (Benemid) par jour à cette dose de PAS. Un accroissement de la concentration sanguine du PAS de 40 à 50% a succédé à l'adjonction de probenecid.

La réduction de la dose de PAS à 8 g. par jour, en maintenant la dose de 2 g. de probenecid, a déterminé des concentrations inférieures à celles produites par la dose quotidienne de 12 g., avec ou sans adjonction de Probenecid, mais elles se rapprochaient des taux obtenus avec le PAS, seul, à la dose de 3 g., quatre fois par jour.

Aucune réaction de toxicité due au probenecid n'a été observée.

Les auteurs suggèrent que les malades ne supportant pas la dose quotidienne de 12 g. de PAS pourraient bénéficier de l'adjonction d'une dose de Probenecid de 2 g. par jour à une dose de PAS, réduite à 8 g. par jour.

Acknowledgments

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Case Reports

CONTRALATERAL RIB FRACTURES DURING THORACOPLASTY[1,2]

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INTRODUCTION

Fractures of the contralateral first and second ribs have been observed in 5 patients immediately after the first or second stages of thoracoplasty. These were not associated with pain and were discovered upon routine roentgenograms obtained postoperatively.

A review of the literature revealed but two articles dealing with this surgical complication. Guggenheim and Cohn (1) studied eight such fractures in 208 patients who had thoracoplasty performed in the National Jewish Hospital, Denver, Colorado. These patients each presented a fracture of the first rib; one also had a similar lesion in the second rib. The patients were all young and were evenly divided between males and females. Seven of the fractures developed after the first stage of thoracoplasty and one after the second. In seven the fracture was complete, and in four of these there was separation of the fragments. In no instance were there such complications as pressure upon the brachial plexus, injury to blood vessels, or perforation of the pleura.

In two cases, roentgenograms obtained immediately after a stage of surgery failed to reveal the fracture which, however, was seen upon a film made seven to ten days later.

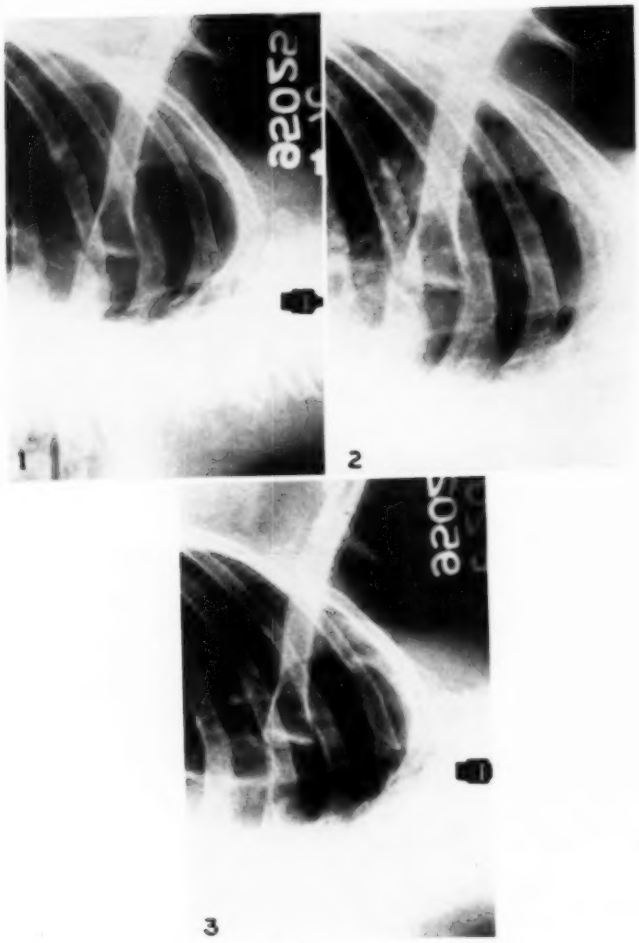
An interesting clinical feature was the absence of pain, despite the presence of a fresh fracture with bony displacement. One of the eight patients had slight pain for a few days. There were no instances of roentgenographic or clinical evidence of prior or subsequent disease of the involved ribs. Healing occurred promptly, with callus formation; indeed, in some cases the lesion was first discovered when the callus was seen upon the films.

In a discussion of the mechanisms by which these fractures may occur, Guggenheim and Cohn mentioned trauma, either applied directly to the rib or indirectly through the clavicle or manubrium. Other possibilities mentioned were fracture due to cough, and prolonged strain due to traction during surgery or to the patient's position on the operating table. As predisposing factors they considered decalcification and general debility following chronic disease with prolonged bed rest.

They concluded that the fractures were probably postoperative in time, based upon the two instances in which no fracture was seen immediately postoperatively but in which the lesion was seen seven to ten days later. They postulate a muscular imbalance which is produced by the removal of the upper ribs on the operated side, allowing the effect of the serratus anterior and the scaleni anterior and medius to be unopposed,

[1] From the Tuberculosis Service, Veterans Administration Hospital, Dayton, Ohio; and the College of Medicine, University of Cincinnati.

[2] Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

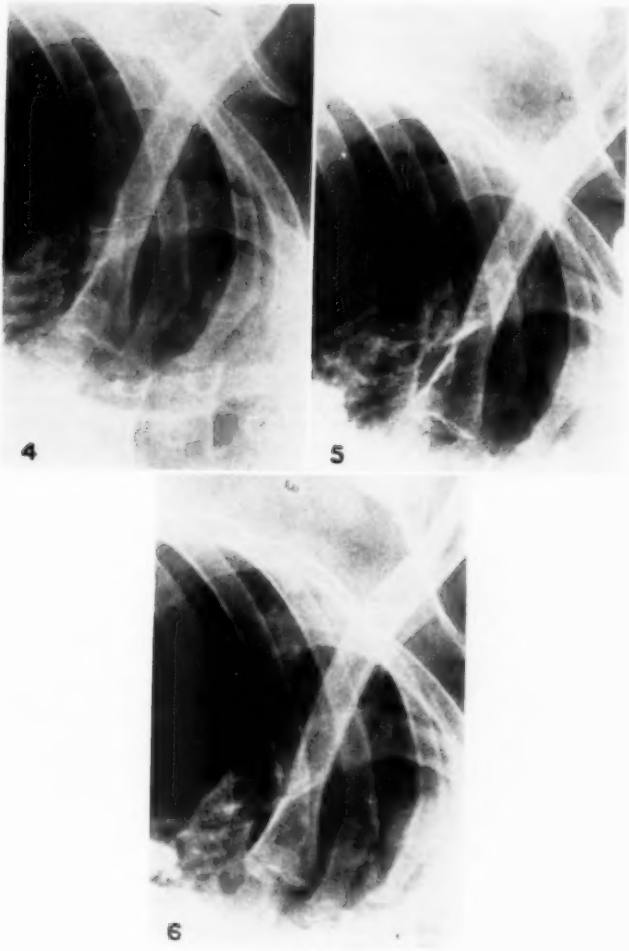


FIGS. 1–3. Case 1

FIG. 1. Detail of right first rib: Roentgenogram of March 13, 1950 (not shown), negative. First-stage (3 ribs) left thoracoplasty, March 15, 1950. Roentgenogram of March 20, 1950 (not shown), negative. Roentgenogram of April 10, 1950, shows incomplete fracture of right first rib.

FIG. 2. Roentgenogram of April 26, 1950, nine days after second-stage left thoracoplasty on April 17, 1950, shows increase in the fracture of the first rib.

FIG. 3. Roentgenogram of June 20, 1950, shows healing with callus formation



FIGS. 4-6. Case 3

FIG. 4. Roentgenogram after first-stage thoracoplasty shows normal right second rib.

FIG. 5. Roentgenogram five days after second-stage thoracoplasty shows complete fracture of right second rib.

FIG. 6. Roentgenogram several weeks later. Callus formation roughly simulates an exudative pulmonary infiltrate.

producing a steady traction upon the contralateral first rib for a period of days or weeks. They point out that all but one fracture occurred in the middle third of the rib, the area of greatest stress and at the same time the weakest portion of the rib due to the presence of grooves for the subclavian artery and vein.

Dingley (2) reported two cases of contralateral fracture of the first rib following thoracoplasty. His experiences were similar to those mentioned above, as well as to our own.

During a period of two years, 5 cases of such fractures have been observed in the Veterans Administration Hospital, Dayton, Ohio. Two illustrative cases will be reported in some detail and 3 others more briefly.

CASE REPORTS

Case 1. F. A. B., a 25-year-old man, had pulmonary tuberculosis from 1947. The first stage (three ribs) of an eight-rib left thoracoplasty was performed on March 15, 1950. The first rib on the right was normal preoperatively (on a roentgenogram of March 13, 1950). No lesion could be seen on the first postoperative roentgenogram of March 23, 1950. The film of April 10, 1950 (figure 1), obtained after an interval when no surgery had been performed, revealed an incomplete fracture of the right first rib. This was not noticed at the time and a second-stage thoracoplasty was performed on April 17, 1950. An increase in the size of the fracture is illustrated in figure 2 (April 26, 1950). Figure 3 (June 20, 1950) shows well-marked callus formation. At no time was any pain associated with this fracture and, if routine roentgenographic observations had not been made, this lesion would not have been detected.

Case 2. W. J. S., a 40-year-old male, had pulmonary tuberculosis from 1945. The first stage of an eight-rib right thoracoplasty was begun on March 20, 1950. Because of some question as to his pulmonary reserve, two ribs only were removed at the first stage. No definite bony lesion could be seen in the postoperative films. The second stage, with the removal of three more ribs, was done March 29, 1950. A roentgenogram of April 10, 1950, showed multiple fracture lines in the left first rib with no separation of the fragments. The lesion was not diagnosed at the time, and a third stage was performed on April 21, 1950. The roentgenogram of May 1, 1950, now showed a fracture of the left first rib with complete separation of the fragments. Although no further surgery was performed, on May 11, 1950, severe left lower chest pain was noticed and a film of May 12, 1950, showed a spontaneous fracture of the left seventh rib. When callus formation was quite marked, evidence of a recent fracture of the left second rib could be visualized. Although the exact date of the actual fracture of this rib could not be ascertained, it apparently occurred at the same time as the fracture of the first rib. At no time was there any pain in the region of the fractured first or second ribs.

Three other cases will be described briefly. In one of these the right second rib only was fractured (figures 4, 5, 6); this lesion developed after the second stage of left thoracoplasty and was unassociated with any symptoms. In the fourth case of the series, a right first-stage thoracoplasty was followed by fractures of the left first and second ribs. The fifth patient had a right thoracoplasty. Films made one and two days postoperatively revealed no fracture, but a third roentgenogram twelve days after the first-stage thoracoplasty presented a linear fracture line in the left first rib. Four days after the second stage a complete fracture could be visualized, with some displacement of the fragments. No symptoms were present in any of these cases and callus formation and healing were prompt in all.

COMMENT

Fractures of the first ribs due to any cause are quite infrequent. Liberson (3) in an analysis of 2,014 rib fractures found only 4 instances involving the first rib and 25 in

which the second rib was broken. In addition he made certain comments about the delayed roentgenographic appearance of fractures of the ribs. He states:

> In 100 cases of recent chest injury suspected clinically of rib fractures and in which roentgen examination was negative, re-examination three weeks later disclosed fractures in 22 cases. . . . In another 100 cases with fractures of one or more ribs, re-examination three to six weeks later disclosed one more fractured rib in each of 32 patients.

Thus in 20 to 30 per cent of rib fractures (as proved by subsequent roentgenographic study) the immediate film may be negative.

Garber (4) reported a single case of fracture of the right first rib which occurred while the subject lifted a weight of approximately 150 pounds. He interpreted this lesion as being due to muscular traction. It was associated with a considerable degree of localized pain. Breslin (5) emphasized the infrequency of fractures of the first rib alone. He analyzed twenty-seven earlier published cases and presented five of his own. All were due to direct or indirect trauma or to heavy weight lifting and were always associated with pain in the neck and shoulder. Breidenbach (6) mentioned the fact discussed above, that fractures of the first rib may be overlooked at the initial roentgenographic examination but were easily detected subsequently when callus formation had developed.

Guggenheim and Cohn found these fractures in 3.8 per cent of their thoracoplasty patients. The 5 cases reported here were seen in a period of two years, during which time thoracoplasty was performed upon 76 patients. Since the fractures occurred after first or second stages of surgery, the incidence, in the present writers' experience, is 6.5 per cent in terms of patients and 4 per cent in terms of operative stages. Thus, it can be seen that these contralateral fractures are fairly common. In 3 cases the fracture followed the first stage of thoracoplasty, and in 2 it became worse during the second. In 2, the fracture followed the second stage and in one of these it became worse during the third stage. In 2, the first rib only was involved, in one case the second alone was fractured, while in the remaining 2 instances, both first and second ribs presented the lesion in question.

In 2 cases in the present series, as in 2 of Guggenheim and Cohn's, roentgenograms made immediately postoperatively showed no fracture, while one could be seen a week or ten days later, with no intervening surgery. This might suggest that in this type of lesion the fracture does not result from direct or indirect trauma at the time of thoracoplasty but may instead be a result of muscular imbalance brought about by temporary loss of muscular action on the operated side. This reasoning may be invalidated, however, by Liberson's (3) finding of the delayed roentgenographic visualization of rib fractures in a percentage approximately equal to that in the present series.

Perhaps a further point favoring the idea that these fractures developed postoperatively is their localization in the first and second ribs. It has been suggested that, with a patient in the lateral decubitus, as during thoracoplasty, and with the complete muscular relaxation of general anesthesia, the patient's own weight plus pressure on the chest by the surgeons may produce sufficient indirect trauma to cause the fracture. While this factor cannot be completely excluded, under such circumstances fractures would be anticipated in the lower ribs (seventh, eighth, ninth, and tenth) which would support part of the body weight, rather than in the first and second ribs which are partly protected by the shoulder girdle.

The actual event leading to the fracture may be cough. Rib fractures due to cough have been seen with considerable frequency (7). Such fractures have always been reported as involving the lower ribs. It is possible that in the present series the muscular

inequality due to the thoracoplasty plus the pain of the recent surgery (causing excessive splinting of the lower ribs) may result in increased strain on the first and second ribs, causing fracture due to muscular traction.

None of the present cases, nor the others reported, showed any evidence of localized involvement of the fractured rib by tuberculosis or other disease processes. Healing occurred promptly in each case. While decalcification of bone, and debility, may play a part in these lesions, there is no roentgenographic or clinical evidence to suggest that this was true; 2 of the 5 patients were robust, rather than frail and asthenic.

The clinical significance of these lesions is probably slight. In no instance were there untoward symptoms, nor was there any involvement of nerves, vessels, or pleura by the bony fragments. In 2 cases, further stages of thoracoplasty were performed after the initial fracture had developed. In both, the fracture became more extensive but no harmful effects were associated with the bony lesion.

The detection of this lesion is somewhat difficult if it is not specifically sought. In none of the 5 cases reported was the fracture diagnosed until after callus formation had developed. In 2 cases, the shadow cast by the callus was at first thought to indicate an exudative spread of tuberculosis, but this error was quickly corrected. In one case, the fractures of the first and second ribs were followed by a painful fracture of the seventh rib, which called attention to the asymptomatic involvement of the two ribs. All these healed promptly.

SUMMARY

Five patients developed contralateral fractures of the first and second ribs during or soon after first or second stages of thoracop'asty. Fractures of this type may be a result of muscle imbalance brought about by the rib resection. The patients experienced no symptoms from the fractures, and the lesions all healed promptly.

SUMARIO

Fracturas Costales Contralaterales Durante la Toracoplastia

Cinco enfermos han manifestado fracturas contralaterales de la primera y la segunda costillas durante el primer o segundo tiempos de la toracoplastia o poco después. Eso puede ser consecuencia del desequilibrio muscular creado por la costectomía. Todos los enfermos fueron asintomáticos y sanaron rápidamente.

RESUME

Fracture contro-latérale de côtes au cours de la thoracoplastie

Chez cinq malades il s'est produit des fractures contro-latérales de la première et de la deuxième côte pendant ou aussitôt après le premier ou le deuxième stade de la thoracoplastie.

Ces accidents peuvent résulter d'un déséquilibre musculaire provoqué par la résection de côtes.

Tous ces cas ont été asymptomatiques et ont guéri rapidement.

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Notes

PURINE ENZYMES IN MYCOBACTERIA

It has been shown that antituberculous drugs, in tuberculostatic concentrations, inhibit the xanthine oxidase of milk[1, 2, 3] and the purine enzymes of animal tissues[4]. The inhibition depends on the concentration of the purine used as substrate. Certain analogues of the purine series inhibit the growth of *Mycobacteria*[5]. It was of interest to determine whether *Mycobacteria* contained purine enzymes and, if so, whether streptomycin and *p*-aminosalicylic acid (PAS) inhibited them. These enzymes have not previously been described in *Mycobacteria*, so a survey of them in several species was made.

M. smegmatis 101, *M. stercoris* 77, *M. BCG* 8240, *M. butyricum* 362, and *M. phlei* 10142 were obtained from the National Type Culture Collection. H37Rv was originally obtained from the Trudeau Laboratories. The organisms were grown on Long's medium with the exception of H37Rv, for which Vorwald's modification of the Proskauer-Beck medium was used. The bacteria were harvested during their logarithmic growth phase, suspended in 0.1 M phosphate buffer pH 7.4, and centrifuged in Hopkins tubes. They were washed once by centrifugation, and then one volume of the packed bacteria was suspended in 20 volumes of the buffer. Four and five-tenths milliliters of this suspension with and without added purines were placed in 50 ml. Erlenmeyer flasks and incubated aerobically two hours at 37°C.; 1.0 ml. of 10 per cent trichloroacetic acid was then added to each flask.

The bacteria were centrifuged down and the following estimations made on the supernate: uric acid by the method of Benedict and Franke[6]; allantoin by the method of Young and Conway[7] (this method actually estimates allantoic acid after hydrolysis of allantoin, therefore estimations before and after hydrolysis gave values for the amount of allantoic acid and allantoin present); urea by the method of Barker[8] unless allantoin was present, in which event the trichloroacetic acid was omitted, urease added, and the ammonia estimated by the method of Speck[9]. The colors were estimated in the Evelyn photocolorimeter with suitable filters.

The results are summarized in table 1. Some generalizations can be made. If, for example, uric acid is produced from xanthine as in *M. smegmatis*, it is metabolized more slowly than added uric acid. This is also true of the urea produced by *M. stercoris* and BCG, in comparison with the urea added. It is possible that a high initial substrate concentration results in a more rapid metabolism. Then the action of the drugs shows some apparent anomalies. For instance, in *M. smegmatis*, streptomycin in a concentration of 1:15,000 inhibited the production of uric acid from xanthine but higher concentrations did not; and in *M. butyricum* only the lowest concentration inhibited uricase,

[1] Di Fonzo, M.: Il Farmaco, 1950, *1*, 6.

[2] Di Fonzo, M., and Di Raimondo, F.: Boll. Soc. ital. biol. sper., 1950, *26*, 1542.

[3] Di Fonzo, M., and Di Raimondo, F.: Boll. Soc. ital. biol. sper., 1951, *27*, 500.

[4] Di Fonzo, M.: in press in Boll. Soc. ital. biol. sper.

[5] Rose, F. L.: Abstracts of papers 120th Meeting Am. Chem. Soc., New York, September, 1951, 5L.

[6] Benedict, S. R., and Franke, E.: J. Biol. Chem., 1922, *53*, 387.

[7] Young, E. G., and Conway, C. F.: J. Biol. Chem., 1942, *142*, 839.

[8] Barker, S. B.: J. Biol. Chem., 1944, *152*, 453.

[9] Speck, J. F.: J. Biol. Chem., 1949, *179*, 1387.

TABLE 1

THE METABOLISM OF CERTAIN PURINES BY SEVERAL SPECIES OF MYCOBACTERIA

ORGANISM	SUBSTRATE ADDED	DRUG ADDED	URIC ACID FOUND	ALLANTOIN FOUND	ALLANTOIC ACID FOUND	UREA FOUND	AMMONIA FOUND
M. smegmatis 101	—	—	4.2	0.7	1.3	18.0	15.0
	Xanthine	—	38.4				
	Guanine	—	5.1				
	Adenine	—	4.2				
	Hypoxanthine	—	4.2				
	Uric acid	—	5.5	0.2	1.3	24.0	30.0
	Allantoin	—		3.5	2.5	22.0	25.0
	Allantoic acid	—			2.5	19.0	28.0
	Urea	—				17.0	27.0
	Xanthine	PAS 1:1,000	26.1				
	Xanthine	1:10,000	30.1				
	Xanthine	1:100,000	32.1				
	Xanthine	SM 1:1,500	38.2				
	Xanthine	1:15,000	29.4				
	Xanthine	1:150,000	39.4				
M. stercoris 77	—	—	4.7	1.8	2.6	7.2	12.2
	Xanthine	—	6.2				
	Guanine	—	5.5				
	Adenine	—	5.1				
	Hypoxanthine	—	4.9				
	Uric acid	—	7.5	5.0	6.5	40.0	5.8
	Allantoin	—		1.2	6.5	46.0	6.1
	Allantoic acid	—			9.0	42.0	8.5
	Urea	—				12.0	40.0
M. butyricum 362	—	—	0.6	1.2	1.4	41.0	
	Xanthine	—	0.8	1.2	1.5		
	Guanine	—	0.8				
	Adenine	—	2.0				
	Hypoxanthine	—	0.8				
	Uric acid	—	2.5	1.2	3.0	53.0	
	Allantoin	—		1.0	1.5		
	Allantoic acid	—			1.9	50.0	
	Urea	—				50.0	
	100 mg. uric acid	—	15.0	5.2	6.7		
	100 mg. uric acid	PAS 1:1,000	40.0	0.0	40.0		
	100 mg. uric acid	1:10,000	44.0		16.0		
	100 mg. uric acid	1:100,000	75.0	6.0	14.0		
	100 mg. uric acid	SM 1:500	28.5	0.0	10.0		
	100 mg. uric acid	1:15,000	26.5	6.2	8.2		
	100 mg. uric acid	1:150,000	64.5	5.2	12.0		

TABLE 1—*Continued*

ORGANISM	SUBSTRATE ADDED	DRUG ADDED	URIC ACID FOUND	ALLANTOIN FOUND	ALLANTOIC ACID FOUND	UREA FOUND	AMMONIA FOUND
M. phlei 10142	—	—	1.0				
	Xanthine	—	5.7				
	Guanine	—	10.4				
	Adenine	—	1.1				
	Hypoxanthine	—	1.1				
	Uric acid	—	61.5				
	Xanthine	—	3.6				
	Xanthine	PAS 1:1,000	5.5				
M. BCG 8240	—	—	1.6	5.5	5.3	36.0	14.0
	Xanthine	—	1.2	2.5	7.4	58.0	12.0
	Guanine	—	2.0	7.4	2.5	64.0	8.0
	Adenine	—	1.2	9.0	3.5	62.0	10.0
	Hypoxanthine	—	0.0	4.5	4.3	43.0	10.0
	Allantoin	—		0.8	6.5	60.0	12.0
	Allantoic acid	—			3.5	58.0	4.0
	Urea	—				40.0	38.0
H37Rv	—	—	2.0				
	Guanine	—	5.6				
	Hypoxanthine	—	1.9				
	300 mg. guanine	—	6.1				
	300 mg. adenine	—	1.3				
	300 mg. hypoxanthine	—	1.2				
	Uric acid	—	63.0				
	Xanthine	—	2.1				
	Xanthine	PAS 1:1,500	3.9				

The results expressed in micrograms represent the amount present in solution after two hours' incubation at 37°C. with the added substrates.

Unless otherwise noted, 60 micrograms of substrate were used.

SM = streptomycin.

whereas the higher ones were less effective. Somewhat analogous concentration effects have been observed in the action of streptomycin on xanthine oxidase of milk, on purine enzymes of animal tissues, and on the growth of *Mycobacteria*[4], but they are difficult to explain. In *M. butyricum*, PAS also showed a greater inhibition of uricase at the lowest concentration used; in some concentrations it increased the oxidation of xanthine by H37Rv and *M. phlei*. Finally, hypoxanthine in comparison with xanthine was either very slowly oxidized or, as was true of H37Rv, inhibited the endogenous uric acid production. In this connection it is of interest that Marshak[10] showed that hypoxanthine inhibited the growth of *M. tuberculosis* H37Rv. *M. smegmatis* and *M. stercoris* oxidized xanthine, guanine, and uric acid, and hydrolyzed allantoin and urea. Adenine was apparently not utilized. On the other hand, *M. butyricum* utilized adenine better than guanine and xanthine. It also oxidized uric acid, hydrolyzed allantoin, but contained

[10] MARSHAK, A.: Am. Rev. Tubere., 1950, *62*, 333.

very little urease. *M. phlei* did not oxidize uric acid and utilized guanine better than xanthine. It had little effect on adenine. BCG utilized uric acid, allantoin, and urea; and the urea values indicate that guanine and adenine were attacked to some extent. Finally, guanine is the only purine metabolized by H37Rv unless the apparent oxidation of xanthine in the presence of PAS is taken into account. It should be pointed out that, in order to form uric acid, both guanine and adenine must be deaminated and then oxidized by the xanthine oxidase. Thus, the differences in the utilization of the two compounds and xanthine by the various organisms must reflect a difference in their ability to penetrate into the cell.

It is apparent that the six species of *Mycobacteria* studied contain certain enzymes which attack purines and urea. Only two, *M. smegmatis* and *M. stercoris*, are able to metabolize xanthine to ammonia. In the others, it has not been possible to demonstrate the complete pathway. This may, however, only reflect the inability of the added substrates to penetrate the cell. The action of streptomycin and PAS on these reactions is complex. Because PAS inhibits certain reactions in the saprophytes, the growth of which is not inhibited by the drug, it may appear that its primary point of attack is not on purine catabolism. On the other hand, the presence of purine enzymes in *Mycobacteria* correlated with the fact that these substances, in tuberculostatic concentrations, are active on such enzymes offers a good reason in explanation of the activity of the drugs on tubercle bacilli by their action on purine enzymes. It is possible to accept the latter assumption on the basis of the following general considerations. The purine catabolism in *Mycobacteria* shows individual characters, since the amounts or the physico-chemical conditions under which the corresponding enzymes are present differ from strain to strain. Therefore, in the chain of enzymatic reactions to which the life of the microorganism is bound, the consequences proceeding from an added substance which is active on purine enzymes will differ from strain to strain. The assumption of Hinshelwood[11] that the division of the cell may occur only when some essential parts of its structure reach a certain critical amount is a suitable development of such considerations.

In summary: The presence of enzymes involved in purine catabolism has been demonstrated in six species of *Mycobacteria*, and the effect of streptomycin and PAS on them has been described. The bacteriostatic effect of these chemotherapeutic agents on the tubercle bacillus has been explained by their effect on purine enzymes.

Acknowledgment

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[11] HINSHELWOOD, C.: Endeavour, 1949, *32*, 151.

COMPLICATIONS FOLLOWING BRONCHOSPIROMETRY[1]

During the past few years the use of bronchospirometry has become increasingly common in the evaluation of the tuberculous patient prior to surgery. The procedure involves the introduction of a catheter in the tracheobronchial tree in patients with active pulmonary tuberculosis. The disease is usually in an unstable phase, and the possibility of affecting the patient deleteriously is of considerable importance. The present study has been undertaken to determine whether any untoward effects could be demonstrated in a large series of patients who have undergone bronchospirometry.

From February 10, 1948, to January 21, 1951, at the Veterans Administration Hospital, Wood, Wisconsin, there were 219 bronchospirometries performed on 177 patients. Of these, the 191 procedures performed on 154 tuberculous patients form the basis for study. The patients were all adult white males.

Premedication consisted of 0.1 gm. of Seconal on the preceding night, 0.1 gm. of Seconal approximately one and one-half hours before, and 75 mg. of Demerol with atropine sulfate 0.0006 gm. twenty minutes before, the procedure was started. Following preliminary anesthesia with 7 cc. of 1 per cent Pontocaine, which was administered by spray and cotton pledgets, a Zavod or Gebauer catheter was introduced into the trachea by the indirect method. In cases in which cough persisted, a small amount of 1 per cent Pontocaine was allowed to run down through the catheter into the left main bronchus. The catheter was allowed to remain *in situ* for six to ten minutes, depending upon the time needed to complete an adequate tracing.

Significant roentgenographic changes were present following 4 of the 191 procedures. One consisted of evacuation of material from a round lesion five days after bronchospirometry. This subsequently refilled and probably represented the usual sequence of events in the natural history of the blocked cavity. There were small infiltrative changes in the chest roentgenograms of 3 patients with bilateral far advanced progressive tuberculosis. The extent of the changes in relation to the pre-existing disease was slight. These appeared at fourteen, eighteen, and twenty-four days following bronchospirometry. Any change which occurred after an interval of longer than one month was arbitrarily excluded from consideration.

Most patients had a transient increase in the quantity of sputum, which disappeared in a few days.

One patient had slight hemoptysis immediately following the bronchospirometry; this became progressively worse, and six weeks later an extrapleural pneumothorax was done in the hope of stopping the hemoptysis. The patient did not survive. Death was due to postoperative complications. The source of hemorrhage was not found.

Fifteen patients had fever of 1°F. or more. In 4 patients the fever appeared immediately following the bronchospirometry; in 11, on the following day. In 3 of the patients there was associated roentgenographic change.

Most of the patients complained of sore throat but in all it was transient in nature and not severe. Occasional patients in whom passage of the catheter was accomplished with great difficulty showed evidence of mild trauma to the larynx.

It appears from analysis of the roentgenographic findings, temperature readings,

[1] Published with permission of the Chief Medical Director, Department of Medicine and Surgery, Veterans Administration, who assumes no responsibility for the opinions expressed or conclusions drawn by the authors.

laryngeal findings, and symptomatology that the incidence of complications following bronchospirometry is low. Since the person who is subject to this procedure is frequently a patient with active pulmonary tuberculosis, sometimes in its most unstable form, it seems reasonable to conclude that the changes which occurred were those which might be expected in all groups such as this. McIndoe and his associates[1] in 1939 studied a group of 272 patients who underwent bronchoscopy. Increased disease was present in four following this procedure. The changes took place four weeks after the procedure had been performed and were considered within the normal expected range of complication.

In summary: A study of the complications following bronchospirometry performed at this hospital was carried out and revealed 4 definite instances of roentgenographic change following 191 bronchospirometries. In 3 of the patients in whom roentgenographic changes occurred, fever was also present. None of the changes was of great magnitude in comparison with the pre-existing disease, and only one was serious. Most of the patients had temporary sore throats. One patient had significant hemoptysis. Fifteen patients had associated transient fever.

It can be concluded that bronchospirometry properly performed is not usually accompanied by severe complications.

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[1] Am. Rev. Tuberc., 1939, *39*, 617.

SIMPLIFIED BRONCHOGRAPHY[1]

Exploration of the bronchial tree using a contrast medium was first introduced by Chevalier Jackson[2] in 1918, more than thirty-three years ago. The value of this procedure in the field of chest diseases has increased in direct proportion to the increasing knowledge and developments in the fields of physiology, anatomy, diagnosis, and treatment of pulmonary diseases.

Bronchography has been of inestimable value in diagnosing, as well as determining, the location and extent of bronchiectasis, thereby aiding the clinician in the intelligent application of dependent drainage and the surgeon in determining the extent of his excisional therapy. The development of segmental pulmonary resection has no doubt been influenced by the bronchographic findings. Bronchograms also aid in defining the precise site of intrapulmonary and intrabronchial lesions or neoplasms. By outlining the bronchial tree, confusing mediastinal densities may be excluded from, or included in, lung tissue.

Clinicians interested in chest diseases are often confronted with patients with tubercle bacilli in the sputum in whom the source of the positive sputum cannot be determined either roentgenographically (including planigraphy) or bronchoscopically. Bronchograms frequently solve these problem cases by revealing an heretofore unsuspected area of bronchiectasis. When the procedure is used preoperatively, the decision whether collapse or excisional therapy is to be performed may be made with greater certainty, thus averting many thoracoplasty failures. Contrariwise, in thoracoplasty patients who continue to discharge tubercle bacilli after operation, bronchograms may reveal an area of tuberculous bronchiectasis more definitely than would be true with planigrams. In this situation also, motheaten areas of rarefaction may be proved to be bronchiectasis by bronchograms.

Bronchograms are not being utilized to their fullest advantage in many tuberculosis hospitals; the procedure being considered to be a last-place diagnostic procedure, if used at all. The fear of complications following the use of iodized oil in active pulmonary tuberculosis patients, such as iodized oil sensitivity, reactivation or spreading of the disease, pleural effusion, and decrease of pulmonary function, has been minimized in the observations of Zavod[3], Robertson and Morley[4], Rabinowitz and Harper[5] and others[6]. Buckles and his associates[7], in 222 bronchographies, had one instance of atelectasis with dyspnea, chest pain, and fever occurring approximately four hours after the oil was introduced. This was relieved by bronchoscopic aspiration. The valuable information gained by bronchograms justifies the procedure in face of the infrequent untoward reactions.

Rather than this fear of complications, the procedure itself appears somewhat for-

[1] This paper was presented, in part, at the regular meeting of the Trudeau Society of Los Angeles, November 27, 1951.

[2] Jackson, C.: Am. J. Roentgenol., 1918, *5*, 454.

[3] Zavod, W. A.: Am. Rev. Tuberc., 1948, *57*, 626.

[4] Robertson, P. W., and Morley, K. D. F.: Lancet, 1951, *1*, 387.

[5] Rabinowitz, P., and Harper, I. S. H.: Dis. of Chest, 1951, *19*, 66.

[6] Dorner, B. A., Friedlander, J., and Wiles, F. J.: Am. Rev. Tuberc., 1944, *50*, 283; 1944, *50*, 287; 1945, *51*, 62; 1945, *51*, 455; 1945, *51*, 519.

[7] Buckles, M. G., Potts, W. L., Davidson, H. B., and Neptune, W. B.: Am. Rev. Tuberc., 1951, *64*, 394.

midable to the occasional bronchographist. The procedure to be presented is so simple that it is probable that it is being used elsewhere. Nevertheless, a limited review of the literature failed to reveal any mention of this technique. Briefly, the procedure consists of introducing the oil through an Abraham cannula, with the patient in the lateral decubitus position, the table tilted five degrees reverse Trendelenburg, and the head supported on a high pillow. The procedure was developed by Dr. Arnaldo Neves[8] and the present writer at the Missouri State Sanatorium, and has been used successfully in more than 250 bronchograms during the past seven years.

The preparation of the patient varies according to the presenting symptomatology. Patients with little or no expectoration need little preparation; those with copious sputum should have dependent postural drainage for variable lengths of time. In some instances, antimicrobial therapy may indirectly decrease the volume of sputum. No food or fluids are allowed for four to six hours prior to the procedure as a precaution against vomiting

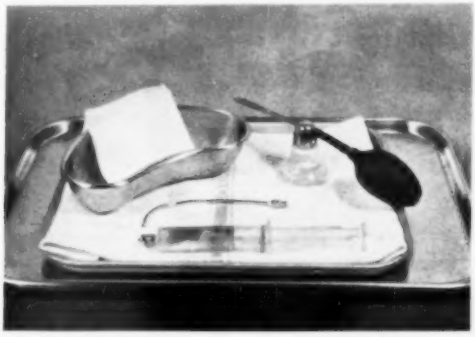

FIG. 1. Equipment used in simplified bronchography

and inhaling food particles. Premedication consists of 0.1 to 0.2 gm. of pentobarbital sodium given one hour before the scheduled time. At the same time, 0.065 gm. of codein and 0.0003 gm. of atropine are given hypodermically to decrease cough and secretions. The patient comes to the radiology department in a wheel chair. On arrival, one drop of the iodized oil is instilled into one conjunctival sac to determine sensitivity of the patient to the oil.

Very little equipment is necessary. As shown in figure 1, the following materials are used:

1 emesis basin.

3 or 4 gauze squares (4 by 4).

1 #15 DeVilbiss atomizer containing 0.5 per cent Pontocaine with or without neosynephrine. A ratio of 1 cc. of 1 per cent neosynephrine to 5 cc. of 0.5 per cent Pontocaine has been found satisfactory. The nasal tip is removed and the movable tip is bent downward approximately 60 degrees.

1 Abraham cannula and a 20 cc. Luer Lok syringe containing warm lipiodol or iodochloral.

[8] Formerly staff physician, Missouri State Sanatorium, Mt. Vernon, Missouri. Present address: Rio de Janeiro, Brazil.

FIG. 2. Method of anesthesia, using a 0.5 per cent Pontocaine spray

FIG. 3. Instillation of the oil in the lateral decubitus position

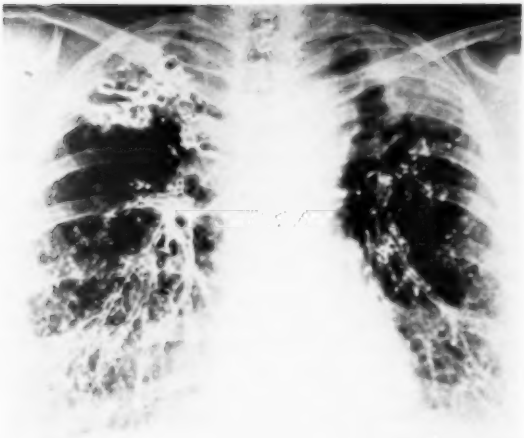

FIG. 4. Bilateral bronchogram, showing saccular bronchiectasis of the right upper lobe and active tuberculous infiltration, left upper lobe.

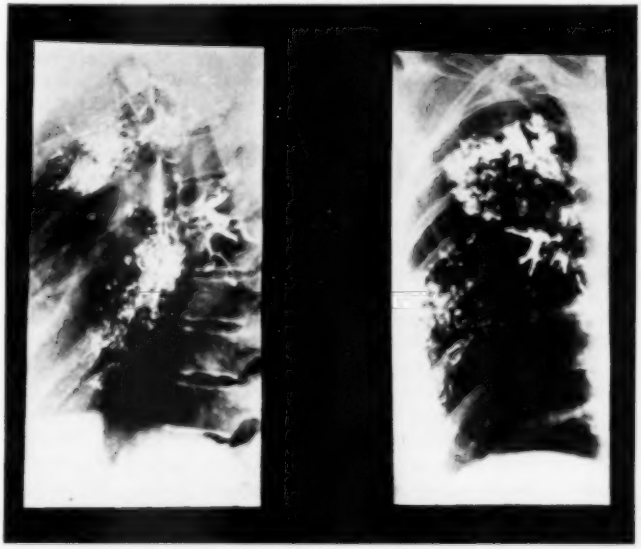

FIG. 5. Selective bronchography, attempting to outline only the upper lobe. Some oil has spilled into the lower branches.

Anesthetization is accomplished by first grasping and withdrawing the tongue, then spraying the posterior pharynx and glottis during phonation with approximately five squirts of the solution (figure 2). After a three- or four-minute interval, the above procedure is repeated, except that this time the patient breathes deeply and the solution is introduced on inspiration. It has been found that sufficient anesthesia results in the average case with five or six inhalations.

Four or five minutes after the last inhalation of the anesthetic, the patient is placed in the lateral decubitus position on the radiographic table with the side to be studied dependent. At this time, the eye can be examined for any evidence of conjunctival injection or edema denoting sensitivity to iodized oil. If sensitivity is present, the procedure is abandoned and repeated later with adequate antihistamine therapy before and after the procedure. The radiographic table is tilted in five degrees reverse Trendelenburg position, the head is propped up on a pillow, the tongue is grasped and pulled forward by the operator, and the cannula is introduced into the upper angle of the mouth and tongue (figure 3). The cannula is used as a retractor in preventing the tongue from drawing back into the pharynx. The patient is instructed to breathe slowly and deeply without coughing or swallowing and, at each inspiration, approximately 0.5 cc. of oil is introduced. When gurgling of oil occurs in the larynx, instillation is temporarily delayed. Approximately 10 cc. of oil is sufficient for one side in the average case, although in large persons, as much as 15 cc. has been required.

After instillation of the oil, the patient is instructed to empty his mouth of excess oil without clearing his throat. Then he is placed in the prone position and instructed to take half a dozen deep breaths. The table may be level or at a 5- to 10-degree Trendelenburg position for filling the upper lobe. The patient then turns over to the supine position for filling the dorsal branches. Fluoroscopy at this time will usually reveal satisfactory filling. Films are taken in the anteroposterior and lateral views, either in the supine or upright position. If desired, the procedure may be repeated on the opposite side and anteroposterior and oblique view films taken.

In figure 4 may be seen the film of a patient with extensive saccular bronchiectasis of the right upper lobe and active tuberculous infiltration of the left upper lobe. Figure 5 demonstrates that by this method selective filling can be accomplished. In this particular case, 7 cc. of oil were introduced to attempt to outline only the upper lobe. However, there has been some spilling into the lower lobe. Perhaps 4 or 5 cc. of oil would have been sufficient.

After the procedure has been completed, the patient is instructed not to cough forcibly but rather to do postural drainage and clear his throat gently to remove the oil. Coughing causes the oil to spread into the alveoli, where it may remain for several months.

In summary: A simplified bronchographic method has been described which consists essentially in instillation of the iodized oil through a cannula with the patient in the lateral decubitus position. It is important that the patient breathe slowly and that the oil be introduced no faster than 0.5 cc. with every inspiration. After simple positioning, an even distribution of the oil occurs throughout the entire bronchial tree.

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American Trudeau Society

Medical Section of the National Tuberculosis Association

Developments in Antimicrobial Therapy of Tuberculosis

A Statement Prepared by the Committee on Therapy

In May, 1951, a committee report was issued on the current status of antimicrobial therapy of tuberculosis[1]. Subsequent developments have produced only a few changes in the use of antimicrobial agents in the treatment of tuberculosis, and the present statement is being issued as a supplement to the earlier report, most of which is still valid.

INDICATIONS FOR ANTIMICROBIAL THERAPY

Since the 1951 report, there has been a trend to broaden further the indications for antimicrobial therapy of tuberculosis to include most patients with active, minimal, pulmonary tuberculosis and tuberculous pleurisy with effusion. In fact, most experts believe that antimicrobial therapy should be used in any case of tuberculosis with active disease unless there is some specific contraindication such as an irresponsible and uncooperative patient or uncontrollable drug allergy.

DURATION OF THERAPY

Studies of the optimal duration of therapy have been carried out only for streptomycin used alone or in combination with para-aminosalicylic acid (PAS). Daily streptomycin alone was effective for only 30 to 120 days, due to the frequent emergence of streptomycin-resistant strains of tubercle bacilli. The administration of streptomycin on only 2 or 3 days of each week combined with the daily administration of PAS has markedly delayed the emergence of drug-resistant bacilli and increased the period of therapeutic effectiveness. Consequently, periods of treatment up to one year and longer are being studied. Preliminary reports indicate that longer periods of treatment are more effective than shorter ones. These data suggest that treatment should be continued for at least 6 to 12 months in every case and that those patients with the more serious types of tuberculosis should be treated 18 to 24 months, possibly longer. Repeated short courses of therapy result in the appearance of drug-resistant strains of tubercle bacilli more frequently than does continuous therapy. Therefore, once treatment is started, it should be continued without interruption until such collateral therapy as collapse or excisional surgery has been completed and until an optimal therapeutic result has been achieved.

CHOICE OF DRUGS

Streptomycin or its derivative, dihydrostreptomycin, combined with PAS remains the most effective drug for the treatment of tuberculosis. Their use is fully described in the 1951 report and the principles described therein are still valid.

Terramycin and viomycin are still being studied but cannot yet be recommended for general use as antituberculous agents. Further studies with amithiozone have revealed it to be hepatotoxic and hematotoxic even in smaller doses, so it should be used with great caution, if at all. Mycomycin has been found to be of no value.

[1] Current status of antimicrobial therapy: Report of the Clinical Subcommittee of Committee on Medical Research and Therapy, American Trudeau Society, Am. Rev. Tuberc., 1951, *63*, 617.

Pyrazinamide is a new drug which is effective against the tubercle bacillus *in vitro* and against experimental tuberculosis in animals. Preliminary reports of clinical studies suggest that it is effective in the treatment of patients with tuberculosis. Unfortunately tubercle bacilli resistant to this drug rapidly appear, thus limiting the period of its effectiveness. Further clinical studies, now in progress, should determine whether or not clinical use of pyrazinamide is warranted.

Isoniazid (isonicotinic acid hydrazide) and its isopropyl derivative, iproniazid (1-isonicotinyl-2-isopropyl hydrazine), recently have been reported to be potent agents against the tubercle bacillus *in vitro* and against experimental tuberculosis in mice, guinea pigs, and rabbits. Preliminary clinical reports indicate that these drugs are of some value in the treatment of patients with tuberculosis. These studies suggest that the optimal oral dose of either drug is 3 to 5 mg. per kg. of body weight per day divided into two or three doses. Few serious toxic reactions have been observed from this dose. Isolated instances of intractable asthma, persistent hypotension, and increased susceptibility to the effects of other drugs (particularly epinephrine, ephedrine, atropine, and demerol) and even deaths have been reported in patients receiving isoniazid or its isopropyl derivative, but the causal relationship has not been clearly established. Minor untoward effects which have been reported are hyperreflexia, constipation, postural hypotension and dizziness, difficulty in starting micturition, eosinophilia, and the appearance of casts, albumin, and reducing substances in the urine. Routine precautions during treatment should include frequent neurologic examinations, blood counts, urinalyses, and tests for renal and hepatic insufficiency. As both drugs are excreted through the kidneys, impairment of renal function may result in the cumulation of toxic concentrations of the drug in the body. For this reason, the renal function should be measured before treatment and, if it is impaired, the dose should be reduced accordingly. Further clinical investigations are needed to determine which of these drugs is better and its exact place in the treatment of tuberculosis. It is already known, however, that tubercle bacilli resistant to these drugs appear rapidly both *in vitro* and in patients. Therefore, there is no reason to believe that isoniazid or its isopropyl derivative will replace the combination of streptomycin and PAS in the treatment of tuberculosis. It seems more likely that these new drugs will be most valuable when used in combination with streptomycin or PAS or both. They may be especially valuable in the treatment of patients with strains of tubercle bacilli highly resistant to streptomycin. In such cases, PAS probably should be given also in an effort to prevent the emergence of isoniazid-resistant strains of tubercle bacilli although the efficacy of this combination has not yet been proved.

SUMMARY

Antimicrobial agents have become indispensable in the treatment of tuberculosis, but should be combined with other appropriate forms of treatment such as rest, collapse therapy, and surgical procedures.

The best regimen available today for most patients with tuberculosis is the combination of 1 gm. of streptomycin or dihydrostreptomycin given intramuscularly two or three times each week plus 12 gm. of PAS given orally, each day.

The optimal duration of therapy with streptomycin and PAS is not known. At least 6 to 12 months of therapy seems advisable; patients with the more serious manifestations of tuberculosis should be treated 18 to 24 months or longer.

Preliminary studies suggest that isoniazid and its isopropyl derivative are potent antituberculosis agents, particularly valuable in the treatment of patients with tubercle

bacilli which are highly resistant to streptomycin. Further studies are needed to determine their proper use in these cases and also in patients with streptomycin-susceptible tubercle bacilli. There is no evidence that either of these drugs is as effective as the combination of streptomycin and PAS.

May 26, 1952

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The Effect of Cortisone and/or Corticotropin on Tuberculous Infection in Man

A Statement Prepared by the Committee on Therapy

In recognition of the growing importance of the effects of cortisone and corticotropin upon tuberculous infection in humans, as the result of scattered reports appearing in the literature in 1950 and 1951, the Committee on Therapy has undertaken to correlate available information on this subject. An interim report of the findings is published at this time in conjunction with a report on the effects of the same agents in experimental tuberculosis, prepared by the Laboratory Subcommittee of the Committee on Medical Research of the American Trudeau Society[1].

An analysis has been made of 81 cases treated with cortisone and/or corticotropin in which known tuberculous infection was present or in which tuberculosis was a complicating disease. The majority of these cases were submitted by physicians from all parts of the country[2] in response to a request from the Committee on Therapy, but a portion of the material was collected from the literature published within the last two years[3].

A summary of the results is given in the accompanying table:

APPARENT EFFECT UPON ASSOCIATED TUBERCULOSIS

HORMONE (S)	METHOD OF EVALUATION	UNFAVORABLE	FAVORABLE	NO EFFECT OR DOUBTFUL EFFECT	TOTAL
		(Number of Cases)			
Corticotropin	Clinical	9	5	7	21
	Autopsy	3	0	1	4
	Sub-total	12	5	8	25
Cortisone	Clinical	17	3	11	31
	Autopsy	9	0	1	10
	Sub-total	26	3	12	41
Cortisone and corticotropin	Clinical	5	2	4	11
	Autopsy	3	0	1	4
	Sub-total	8	2	5	15
Totals	Clinical	31	10	22	63
	Autopsy	15	0	3	18
	Sub-total	46	10	25	81

[1] The effect of cortisone and/or corticotropin on experimental tuberculous infections in animals: Statement prepared by the Laboratory Subcommittee of the Committee on Medical Research (Am. Rev. Tuberc., 1952, *66*, 257).

[2] The Committee wishes to express its indebtedness to the following physicians who were

It will be seen that in 46 cases receiving hormone therapy the course of tuberculous infection was unfavorable; in 10 cases the course was favorable; and in 25 cases no change in the course was evident. It should be recognized that these figures have no statistical validity as an index of the frequency of exacerbation of tuberculous disease by cortisone and corticotropin. A large number of patients with unsuspected tuberculous disease must have received these hormones without harmful effect. The figures do indicate, however, that exacerbation of tuberculous infection may occur during or after treatment with cortisone or corticotropin.

In analyzing the data, it often was difficult to determine if exacerbation of tuberculous disease occurred during or after hormone therapy, and in the table no attempt has been made to separate the two groups. Exact data were received in a number of cases, however, which indicated that serious exacerbation of tuberculosis can occur during hormone therapy.

The Committee on Therapy plans to continue this study, and will welcome having additional cases called to its attention. Clinical abstracts and suitable roentgenograms may be sent to Dr. Robert H. Ebert, University of Chicago, 950 East 59th Street, Chicago 37, Illinois.

CONCLUSIONS

In the analysis of the 81 cases, it is obvious that exacerbation of tuberculous infection does not inevitably result from treatment with cortisone or corticotropin. On the other hand, it seems clearly demonstrated that hormone therapy may have a devastating effect on active or apparently inactive tuberculosis. This was particularly apparent in the cases coming to autopsy. Often there was a marked and even spectacular dissemination of the disease, and the observation was repeatedly made that tubercles were poorly walled off and contained numerous tubercle bacilli.

---

kind enough to cooperate in this study by making their cases available for study and analysis:

L. K. Alpert, Washington, D. C.; T. F. Barrett, Los Angeles, Cal.; P. B. Beeson, Atlanta, Ga.; R. F. Bradley, Boston, Mass.; E. V. Bridge, Cannon Falls, Minn.; A. W. Capon, Weston, Ontario; K. A. Carroll, Hines, Ill.; M. F. Cummings, Chamblee, Ga.; R. Davies, Seattle, Wash.; R. J. Erickson, Albany, N. Y.; C. J. Jones, New Orleans, La.; W. M. Kirkendall, Iowa City, Iowa; M. R. Lichtenstein, Chicago, Ill.; F. J. Lovelock, Bronx, N. Y.; J. W. Middleton, Galveston, Texas; H. J. Moersch, Rochester, Minn.; F. S. North, Palo Alto, Cal.; W. M. Peck, McCain, N. C.; C. Ragan, New York, N. Y.; S. J. Shipman, San Francisco, Cal.; N. P. Shumway, Cleveland, Ohio; M. J. Small, Brooklyn, N. Y.; R. G. Sprague, Rochester, Minn.; H. S. Van Ordstrand, Cleveland, Ohio; L. Wallner, Chicago, Ill.; F. M. Willett, San Francisco, Cal.; J. W. Woods, Durham, N. C.; J. C. Yockey, Long Island City, N. Y.

[3] a. Doerner, A. A., *et al.*: Am. Rev. Tuberc., 1951, *64*, 564.

b. Fred, L., *et al.*: J. A. M. A., 1951, *147*, 242.

c. King, E. A., *et al.*: J. A. M. A., 1951, *147*, 238.

d. Kleinschmidt, R. F., and Johnson, J. M.: Ann. Int. Med., 1951, *35*, 694.

e. LeMaistre, C. A., *et al.*: J. Clin. Investigation, 1951, *30*, 445.

f. Rothman, P. D.: J. Pediat., 1951, *39*, 237.

g. Popp, C. G., *et al.*: J. A. M. A., 1951, *147*, 241.

h. Small, M. J.: Quart. Progress Report V. A., Army, and Navy on Chemotherapy of Tuberculosis, April, 1952, p. 39.

i. Straus, B., *et al.*: Am. J. Med., 1952, *12*, 170.

From the analysis of the clinical material reviewed, the following conclusions appear to be justified:

1. Cortisone and corticotropin therapy may cause serious exacerbation of active or apparently inactive tuberculosis. This is not an inevitable consequence of hormone therapy, but there is no accurate way of predicting the outcome in any individual case.

2. Cortisone and corticotropin therapy, by reducing the inflammatory response to infection, tends to cause poor localization of tuberculous disease and to facilitate widespread dissemination.

3. The development or progression of excavation in pulmonary tuberculosis during or after hormone therapy is fairly commonly noted.

4. Cortisone and corticotropin therapy may reduce the signs and symptoms of tuberculous disease even when the net effect of the therapy is harmful.

5. The exacerbation of old apparently inactive tuberculosis is particularly likely to occur in debilitating diseases such as lupus erythematosus or leukemia.

RECOMMENDATIONS

In view of the above observations and conclusions, the Committee on Therapy feels justified in making the following recommendations for the guidance of physicians within and without the Society:

1. At this time cortisone and corticotropin appear to have no place in the treatment of active tuberculosis as such.

2. In the presence of active tuberculous infection, therapy of other concomitant conditions with cortisone or corticotropin is to be regarded always as potentially hazardous.

3. The presence of old apparently inactive tuberculosis is not of itself an absolute contraindication to hormone therapy, but such a condition should be evaluated meticulously as to possible activity before beginning hormone therapy, and frequent follow-up examinations by roentgenogram and by sputum examination are mandatory.

4. Even in the absence of known tuberculous infection, roentgenographic examination of the chest before, during, and after hormone therapy is wise, since there may be exacerbation of unsuspected tuberculous lesions. This is particularly the case in the treatment of such debilitating diseases as lupus erythematosus and leukemia.

5. If a pulmonary infiltrate appears during or after hormone therapy, pulmonary tuberculosis should be suspected until proved otherwise.

6. Situations will arise in which there is an indication for treatment with cortisone or corticotropin of a nontuberculous condition even in the presence of active tuberculous disease. In such instances it would seem logical to combine hormone therapy with the use of potent antimicrobial agents such as streptomycin and para-aminosalicylic acid (PAS).

May 26, 1952

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The Effect of Cortisone and/or Corticotropin on Experimental Tuberculous Infections in Animals

A Statement Prepared by the Laboratory Subcommittee of the Committee on Medical Research

Approximately thirty-nine published papers and personal communications[1] dealing with the effect of cortisone or corticotropin on experimental tuberculosis have been reviewed. Striking diversities in the results obtained and the conclusions drawn by different workers were noted. In many instances completely opposite effects were described, and even in experiments performed in the same laboratory a lack of uniformity in results was evident. The same diversity of findings was obtained with combinations of the hormones and antibacterial drugs.

It was the opinion of the Committee that much more experimental work was necessary before any final statements could be made regarding cortisone and corticotropin in experimental tuberculosis. Results obtained so far indicate that the effects produced by the hormones depend a great deal on the exact conditions of the experiment, such as species of animal, type of infection, dosage of hormone, and duration of therapy. At the present time, the following are the only statements that seem to be warranted by the available substantiated data:

1. The administration of cortisone or corticotropin to animals with experimental tuberculosis may produce a variety of effects, depending on the species of animal and the experimental conditions.

2. The hormones produce a lessened reactivity of the small blood vessels, which, in turn, causes a reduction in the inflammatory response of sensitized tissue to the tubercle bacillus.

3. Cortisone can convert a chronic smoldering tuberculous infection in mice into an acute progressive process.

4. When the adrenal hormones are withdrawn, there may be a sudden flare-up of tuberculous disease.

5. The hormones have no significant action on the tubercle bacillus or tuberculin *in vitro*.

May 26, 1952

William E. Dye
Gladys L. Hobby
Gardner Middlebrook
Frank Princi
Guy P. Youmans

William Steenken, Jr., *Chairman*

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NOTES

ANNUAL MEETING OF AMERICAN TRUDEAU SOCIETY—1953

The American Trudeau Society, medical section of the National Tuberculosis Association, invites the submission of abstracts of scientific papers for presentation at its next annual meeting in Los Angeles, California, May 18–22, 1953, at the Hotel Statler.

Full information can be obtained by writing to the Chairman of the Medical Sessions Committee, American Trudeau Society, 1790 Broadway, New York 19, N. Y.

SPANISH TRANSLATION OF "DIAGNOSTIC STANDARDS AND CLASSIFICATION OF TUBERCULOSIS"

Diagnostic Standards and Classification of Tuberculosis of the National Tuberculosis Association 1950 has been translated into Spanish under the direction of Professor H. Orrego Puelma of the University Clinic on Phthisiology of the Hospital del Salvador, Santiago de Chile, with the cooperation of a committee appointed by the Union of Latin-American Societies for Tuberculosis. Members of Latin-American societies and others wishing copies are requested to communicate directly with Professor H. Orrego Puelma.

TRADUCCIÓN AL ESPAÑOL DE LAS "NORMAS DE DIAGNOSTICO Y CLASIFICACIÓN DE LA TUBERCULOSIS—EDICIÓN DE 1950"

(Publicaciones Conjuntas de la Catedra de Tisiología del Profesor H. Orrego Puelma y de la Escuela de Salubridad)

El manual *Normas de Diagnóstico y Clasificación de la Tuberculosis* de la Asociación Nacional contra la Tuberculosis de los E. U. A., 1950, ha sido traducido al español bajo la dirección del Profesor H. Orrego Puelma, de la Clínica Universitaria de Tisiología del Hospital del Salvador, de Santiago de Chile, con la cooperación de una comisión designada por la Unión de Sociedades Latino-Americanas de Tuberculosis. Los miembros de dichas Sociedades y otros interesados en adquirir ejemplares tengan a bien comunicarse directamente con el Profesor Orrego Puelma a la dirección mencionada.

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Recent studies of dietary practices among persons from 40 to 75 years of age suggest that protein deficiency is a most likely and a highly significant deficiency found in elderly people.[2,3] In a study concerned with nitrogen balance in healthy elderly men,[4] it was shown that diets containing 0.5 Gm. of protein per kilogram of body weight were incapable of maintaining the subjects in nitrogen balance. Increase of the protein to 0.7 Gm. established equilibrium. Since young adults usually maintain nitrogen equilibrium on a protein intake of 0.5 Gm. of protein per kilogram of body weight, this study indicated that aging adults may require at least as high or a higher protein intake for maintenance of nitrogen equilibrium.[1]

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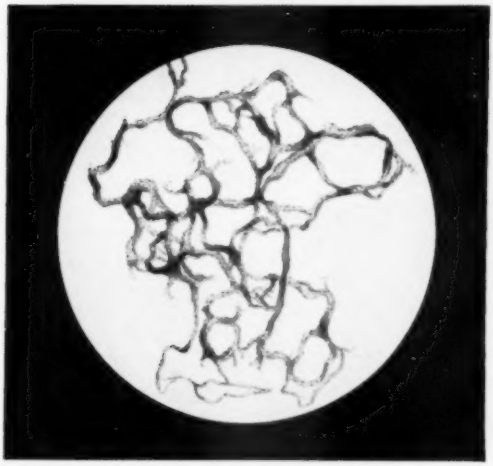

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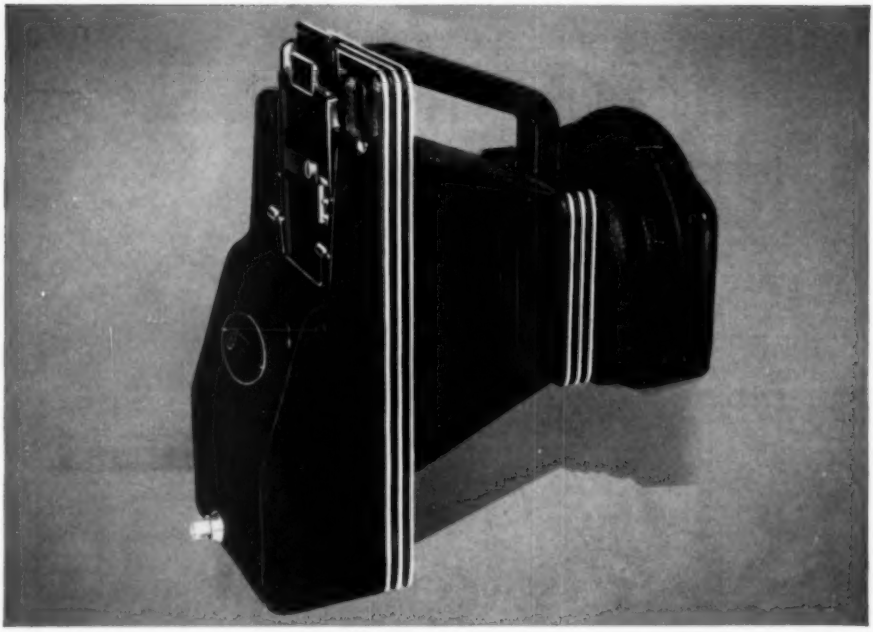

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